IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Patent Application of: Hans Klingemann			
Conf. No.:	5420	Group Art Unit:	1644
Appln. No.:	10/008,955	Examiner:	Ronald B. Schwadron
Filing Date:	December 7, 2001	Attorney Docket No.:	06-129 PCT/US/CIP

Title: NATURAL KILLER CELL LIENS AND METHODS OF USE

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

December 9, 2009

AMENDED APPEAL BRIEF PURSUANT TO 37 CFR § 41.37

Applicant regrets that the Appeal Brief submitted on November 16, 2009, was found defective for the reasons set forth in the Notification of Non-Compliant Appeal Brief dated December 1, 2009. The Appeal Brief was complete in most all respects, but did not contain proper headings required pursuant to 37 CFR 41.37(c). Specifically, Applicant's Appeal Brief erroneously labeled the "Related Appeals and Interferences" section with the heading "Statement of Related Cases." Accordingly, the Appeal Brief has been amended to comply with the requirements set forth in 37 C.F.R. § 41.37(c) to include the heading "Related Appeals and Interferences."

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	)	
Hans Klingemann	)	
	)	
Serial No. 10/008,955	)	**NATURAL KILLER CELL LINES AND METHODS OF USE**
	)	
Filed: December 7, 2001	)	
	)	
Art Unit: 1644	)	
	)	
Patent Examiner: Ronald B. Schwadron	)	
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November 16, 2009

APPEAL BRIEF PURSUANT TO 37 C.F.R. § 41.37

Pursuant to 37 C.F.R. §§ 41.31 and 41.37, Applicant hereby submits the following brief to the U.S. Patent and Trademark Office ("PTO") Board of Appeals and Interferences ("the Board") in support of Applicant's appeal of the Examiner's decision in the final Office Action mailed on March 24, 2009, ("Final Office Action") to finally reject claims 20, 22, 26, 27, and 30. For at least the reasons set forth herein Applicant respectfully submits that the claims as currently presented are patentable and requests that the Board reverse the Examiner's final rejection thereof, remand this proceeding to the Examiner, and order the Examiner to issue a notice of allowance.

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TABLE OF AUTHORITIES

Cases

Statutes

Other Authorities

I. REAL PARTY IN INTEREST

The subject application has been assigned to ZelleRx Corporation who is the real party in interest.

II. RELATED APPEALS AND INTERFERENCES

The following cases are related to U.S. Patent Appn. No. 10/008,955 ("the '955 Application"), currently on appeal:

(1) U.S. Patent Application No. 10/701,359, filed on November 4 2003, entitled "Methods of Treating Tumors Using Natural Killer Cell Lines," which is a divisional of the '955 Application, which is a continuation-in-part of U.S. Patent Application No. 09/403,910, filed on October 27, 1999, now abandoned, which is a national phase entry of PCT/US98/08672, filed on April 30, 1998 and which claims priority to U.S. Provisional Application No. 60/045,885, now expired, filed on April 30, 1997.

(2) U.S. Patent Application No. 10/456,237, filed on June 6, 2003, entitled "Interleukin-Secreting Natural Killer Cell Lines and Methods of Use," which is a divisional of the '955 Application, which is a continuation-in-part of U.S. Patent Application No. 09/403,910, filed on October 27, 1999, now abandoned, which is a national phase entry of PCT/US98/08672, filed on April 30, 1998 and which claims priority to U.S. Provisional Application No. 60/045,885, now expired, filed on April 30, 1997.

III. STATUS OF CLAIMS

Claims 20, 22, 26, 27, and 30 are currently pending. Claims 1-19, 21, 23-25, 28, 29, and 31 are withdrawn from consideration. For convenience, the complete text of the claims is

attached hereto in the Claims Appendix in Section VIII hereto. Claims 20, 22, 26, 27, and 30 were finally rejected and are on appeal:

(a) Claims 20, 22, 26, 27, and 30 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 30-35, 46, 48, 50, and 53 of co-pending U.S. Appn. No. 10/701,359 ("the '359 Application"). Applicant indicated in the Request for Continued Examination filed on October 15, 2008 that a Terminal Disclaimer will be filed upon recognition of allowable subject matter.

(b) Claims 20, 22, 26, 27, and 30 are rejected pursuant to 35 U.S.C. § 103(a) as being unpatentable over Gong et al., *Leukemia* 8:652-658, 1994 ("Gong et al.") in view of U.S. Patent No. 5,272,082 to Santoli et al. ("Santoli et al.").

IV. STATUS OF AMENDMENTS

Applicant filed an amended claim set with the Request for Continued Examination filed on October 15, 2008[1]. Applicant has not filed any amendment subsequent to the Final Office Action mailed on March 24, 2009.

Applicant filed the Notice of Appeal on September 15, 2009.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 20 is directed to a method of treating a pathology *in vivo*. See the '955 Application as originally filed, pg. 1, lines 8-10 (hereinafter, "the '955 Appn., __:__"); see also the '955 Appn. 4:24-26; 5:9-11; 8:9-26; 13:11-20; 15:29-16:5; 16:19-24; 23:24-24:2; 26:18-22; 35:12-25; 39:3-10; 39:19-22; 41:26-42:5; 43:22-29; 44:12-28;

46:24-27; Table 1; Table 5; Table 6; Figure 8; and Figures 11-13). The method is carried out in a mammal. See the '955 Appn., 8:10; 13:16; 18:23-26; 23:24-25; 41:26-30; and 44:1-46:27. Applicant developed a unique cell line identified as NK-92 and available from American Type Culture Collection (ATCC) as Deposit No. CRL-2407. The claimed method comprises the step of administering to the mammal a medium comprising NK-92 cells. See the '955 Appn., 1:10; 8:11; 13:17-18; 14:4-5; 16:11-25; 23:25; and 25:10-12.

Dependent claim 22, which depends from claim 20, is directed to a method wherein the pathology is a cancer. See the '955 Appn., 1:9; 4:25; 5:10; 13:11-13; 23:23-24:2).

Dependent claim 26, which depends from claim 20, is directed to a method wherein the cells are administered to the mammal intravenously. See the '955 Appn. 8:21-22; 23:26-27; 23:29-24:2). The mammal is a human. See the '955 Appn. 8:21-22.

Dependent claim 27, which depends from claim 21, further comprises the step of administering to the mammal a cytokine that promotes the growth of the NK-92 cells. See the '955 Appn., 8:21-24; 24:13-23).

Dependent claim 30, which depends from claim 22, is directed to a solid tumor cancer. See the '955 Appn., 23:29.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1) The rejection of claims 20, 22, 26, 27, and 30 pursuant to 35 U.S.C. § 103(a) as being unpatentable over Gong et al. in view of Santoli et al. is being appealed.

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[1] The Request for Continued Examination was refiled on January 15, 2009 in the Response to Notice of Non-Compliant Amendment to correct the status identifiers of claims 23 and 31 that were improperly labeled as

2) The rejection of claims 20, 22, 26, 27, and 30 on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 30-35, 46, 48, 50, and 53 of co-pending Application No. 10/701,359 is not being appealed. A terminal disclaimer will be submitted upon an indication of allowability of the pending claims.

3) The Examiner's refusal to enter the Substitute Specification on the grounds that it does not conform to 37 C.F.R. § 1.125(b), (c) because it allegedly contains new matter is not being appealed. Applicant will cancel the alleged new matter upon an indication of allowability of the pending claims.

4) The objection to the amendment filed on October 15, 2008 pursuant to 35 U.S.C. § 132(a) on the grounds that new matter is introduced into the disclosure is not being appealed. As set forth above, Applicant will cancel the new matter upon an indication of allowability of the pending claims.

VII. ARGUMENT

A. Introduction

This appeal is based on Applicant's belief that the Examiner has failed to establish a *prima facie* case of obviousness on which to base the current rejections of the claims pursuant to 35 U.S.C. § 103(a). The Examiner's rejection is erroneously premised on the combination of two references, namely Gong et al. in view of Santoli et al., despite the fact that the combination of references fails to teach or suggest Applicant's method of treating a pathology *in vivo* comprising the step of administering to the mammal a medium comprising NK-92 cells. Further support for the lack of obviousness is found in the Declaration of Hans Klingemann, M.D.,

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"withdrawn – previously presented" rather than "withdrawn."

Ph.D., pursuant to 37 C.F.R. § 1.132 (hereinafter, "Klingemann Decl."), the sole inventor of the NK-92 cells disclosed in the '955 Application and a skilled artisan in the fields of translational research, transplantation biology, and tumor immunology. Klingemann Decl., ¶ 14.

Gong et al. disclosed the NK-92 cell line, an immortal cell line originally obtained from peripheral mononuclear cells of a fifty-year-old male patient having non-Hodgkin's lymphoma. Klingemann Decl., ¶ 21; '955 Appn., 14:4-5. At the time that the NK-92 cell line was discovered, the inventor thought that the cell line provided a suitable model to study the biology of NK-cell and activated NK-92 cells. Klingemann Decl., ¶ 22. Gong et al. merely set out to characterize the NK-92 cell line for use as a research tool. Gong et al., Abstract.

Further research with the NK-92 cell line revealed surprising and unexpected results, and it is these further inventions that are claimed in the '955 Application. '955 Appn., 43:22-29. Of particular surprise was the finding that NK-92 cells have cytolytic activity *in vitro* and tumor-inhibiting activity *in vivo*. '955 Appn. 43:22-29. Specific data demonstrating the cytoxic activity of the NK-92 cells are set out in Tables 5 and 6 and Figure 9 of the '955 Application, reproduced below.

Table 5. Cytotoxicity of NK-92, T-ALL104 and YT Clone to Patient-Derived Leukemic Cells[a]

Patient	Disease Status	Blast (%) in Sample	Cytotoxic Sensitivity		
			NK-92	TALL-104	YT
AML					
1 M4□	Relapse	PB (66%)	++++++	+++++	-
2 (M1)	Relapse	PB (50%)	+++++	-	-
3 (M3)	Relapse	PB (50%)	+++ (++++)	+ (++++)	- (-)
4 (M4)	Refractory	PB (90%)	++ (++)	- (+)	- (-)
5 (M2)	New	BM (90%)	+++ (+++)	+ (+++)	ND
6 (M4)	New	BM (97%)	-	-	-
7 (M4)	New	PB (39%)	- (-)	- (++)	- (-)
8 (M3)	New	PB (55%)	- (++)	- (+++)	+ (-)
9 (M3)	New	BM (32%)	-	-	-
T-ALL					
1	Relapse	BM (98%)	++++++	-	-
2	Relapse	PB (85%)	++++++	- (-)	+++ (+++)
3	Relapse	PB (77%)	++++++	- (+)	- (-)
4	Relapse	PB (60%)	+++++	- (-)	+ (-)
5	New	BM (40%)	+++	-	-
6	New	BM (66%)	+++	-	-
B-Lineage-All					
1 ●	Relapse	BM (78%)	+++++	++++	-
2	New	BM (30%)	++++	ND	ND
3	Relapse	BM (75%)	+++ (++++)	+ (++++)	++ (++)
4	New	BM (97%)	++ (+++)	+ (+++)	- (-)
5	Relapse	BM (60%)	+ (+)	- (+)	- (-)
6	Relapse	BM (80%)	-	ND	ND
7	Relapse	PB (80%)	-	- (-)	-
8	New	BM (68%)	-	-	-
9	New	BM (33%)	-	- (+)	-

Patient	Disease Status	Blast (%) in Sample	Cytotoxic Sensitivity		
			NK-92	TALL-104	YT
10	Relapse	BM (87%)	-	- (++)	-
11	Relapse	BM (75%)	- (+++)	- (++++)	-
12	New	BM (30%)	-	-	ND
13	New	PB 90%)	- (+++)	- (+++)	ND
14	New	BM (81%)	-	-	ND
CML					
1	BC	PB (45%)	++++++	+++++	+++
2	AC	PB (22%)	++++++	++	-
3	BC	PB (93%)	+++++	+	-
4	CP	PB (15%)D	++++	+	-
5	CP	PB (8%)D	++ (++++)	ND	ND
6	CP	BM (12%)D	+ (+++)	+ (+)	ND
7	CP	BM (10%)D	+ (+++)	+ (++++)	ND
8	BC	PB (60%)	+	-	-
9	BC	BM (48%)	+	- (-)	-
10	CP	PB (21%)D	+ (++)	- (++++)	- (-)
11	CP	PB (11%)D	-	- (+++++)	- (-)

Notes and Abbreviations. a) Columns show results of chromium release assays at E:T = 9:1 after 4 h without parentheses, and (results after 18 h enclosed in parentheses); New: newly diagnosed; ND: none done; o: FAB classification; D:blast and promyelocyte; BM: bone marrow; PB: peripheral blood; I:B-ALL; BC: blast crisis; AC: accelerated phase; CP: chronic phase.

Table 6. Specific Lysis of Human Leukemia Cell Lines by Natural Killer Cell ClonesNK-92, TALL-104, and YT.

Target	Specific Lysis (%)								
	NK92			TALL-104			YT		
	Effector:Target Ratio								
	9:1	3:1	1:1	9:1	3:1	1:1	9:1	3:1	1:1
K562	94.1	91.2	82.1	88.5	85.2	72.5	34.2	28.2	18.4
HL60	87.9	75.3	79.6	43.0	16.0	6.9	2.1	1.1	1.5
KG1	64.6	53.8	43.7	2.7	0.5	0	0.1	0	0
NALM6	72.6	56.8	52.4	67.8	55.6	33.3	1.0	0.5	0
Raji	86.0	75.4	70.0	22.2	10.2	0.3	25.1	18.0	14.2
TALL-104	57.3	53.2	44.1	-	-	-	3.2	1.4	0.9
CEM/S	56.6	48.8	34.7	2.7	1.6	0.9	0.9	0.4	0.3
CEM/T	57.5	42.1	39.1	1.5	0.6	0.3	1.2	0.1	0.2

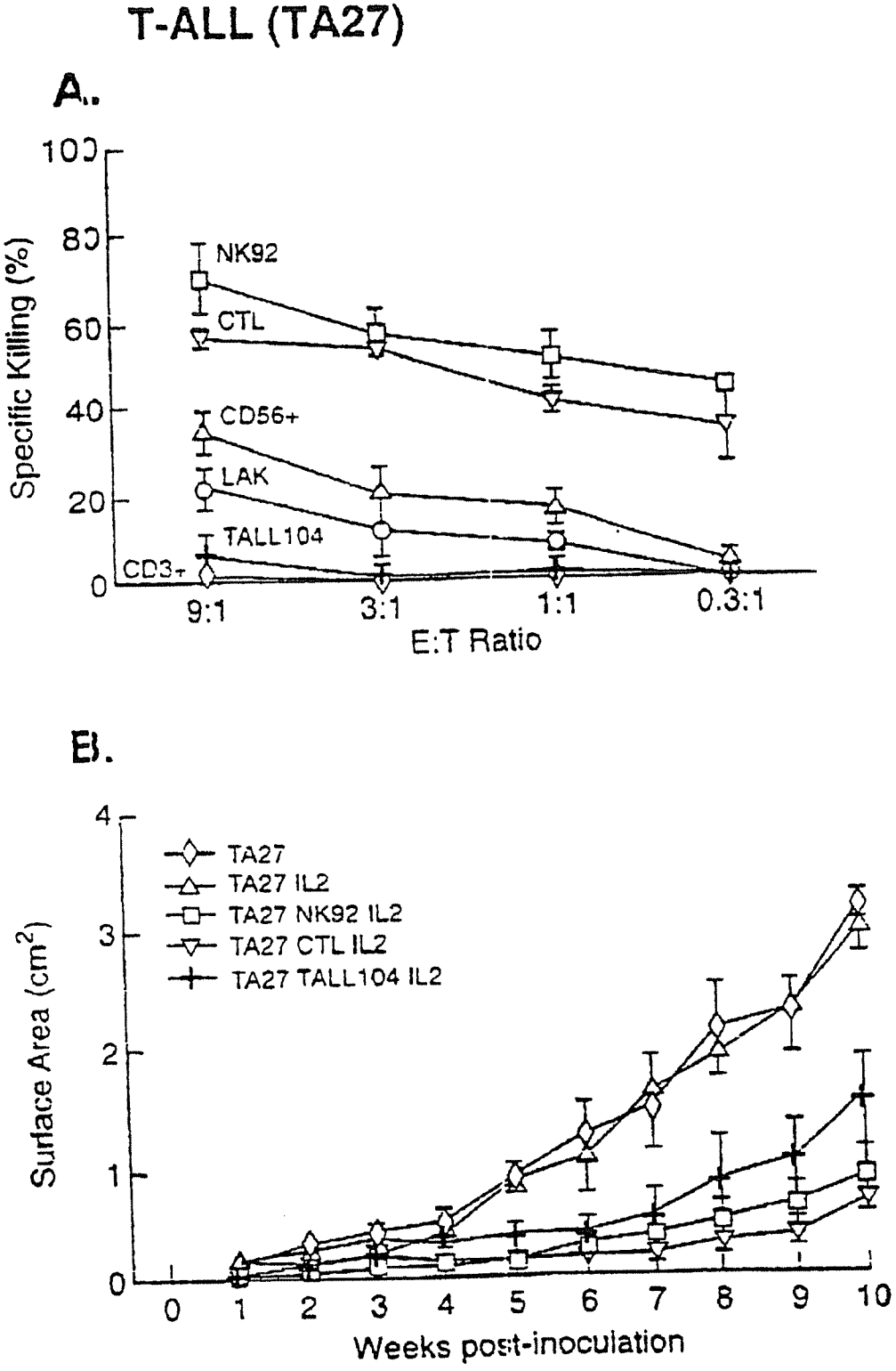
T-ALL (TA27)
A.
Specific Killing (%)
100
80
60
40
20
0
NK92
CTL
CD56+
LAK
TALL104
CD3+
9:1
3:1
1:1
0.3:1
E:T Ratio
B.
Surface Area (cm²)
4
3
2
1
0
TA27
TA27 IL2
TA27 NK92 IL2
TA27 CTL IL2
TA27 TALL104 IL2
0 1 2 3 4 5 6 7 8 9 10
Weeks post-inoculation

FIGURE 9

In contrast to the teachings of Gong et al., Santoli et al. teach "genetically modified cytotoxic T lymphoblastic leukemia cell lines (T-ALL), and uses of these cell lines in cancer therapy." Santoli et al., Abstract. There is absolutely no teaching or suggestion of Applicant's claimed method in either Gong et al. or Santoli et al., alone or in combination. As such, the Examiner's rejection of claim 20 and claims 22, 26, 27, and 30 depending therefrom is completely unsubstantiated. The rejection should be reversed.

B. Applicant's Claimed Method of Treating a Pathology *In Vivo*

Unlike anything shown in the combination of Gong et al. and Santoli et al., Applicant's independent claim 20 claims a method of treating a pathology *in vivo* by administering a medium comprising a particular line of NK-92 cells.

Dependent claim 22, which depends from claim 20, is for a method of treating a cancer.

Dependent claim 26, which depends from claim 20, provides that the NK-92 cells are administered to the mammal intravenously and that the mammal is a human.

Dependent claim 27, which dependents from claim 20, further comprises the step of administering to the mammal a cytokine that promotes the growth of the NK-92 cells.

Dependent claim 30, which depends from claim 22, limits the cancer to a solid tumor.

C. For the reasons explained herein, the Examiner cannot properly rely on the combination of Gong et al. and Santoli et al. to support a rejection of the claims pursuant to 35 U.S.C. § 103(a).

C. Independent claim 20 is not obvious over Gong et al. in view of Santoli et al. because claim 20 recites subject matter not shown or suggested by the cited prior art.

Pursuant to 35 U.S.C. § 103(a), the Examiner has finally rejected claims 20, 22, 26, 27, and 30 directed to a method of treating a pathology *in vivo* in a mammal comprising the step of administering to the mammal a medium comprising NK-92 cells (available from ATCC as Deposit No. CRL-2407) as being unpatentable over Gong et al. in view of Santoli et al.

Specifically, the Examiner alleges that

> Gong et al. teach use of NK-92 cells to lyse leukemic tumor cells. Gong et al. teach that said cells require IL-2 to function. Gong et al. does not in vivo use of NK-92 cells to treat cancer [sic]. Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease or in preclinical in vivo studies. Santoli et al. teach that said cells are injected iv wherein injection utilizes a syringe and wherein the injected NK-92 cells would be adjacent to leukemic cells in the blood. Santoli et al. disclose that the cells can be administered with the cytokine IL-2. Santoli et al. teach that said cells can be modified to bind solid tumors. It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have created the claimed invention because Gong et al. teach use of NK-92 cells to lyse tumor cells, while Santoli et al. teach in vivo use of cytotoxic cell lines. One of ordinary skill in the art would have been motivated to do so because Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease or in preclinical in vivo studies.

Final Office Action, ¶ 8. Applicant disagrees for at least the reasons set forth below.

1. Disclosure of Gong et al.

Gong et al. established the existence of the immortal cell line, NK-92, and set out to characterize the NK-92 cell line for use as a research tool, concluding that the NK-92 cell line "may provide a suitable model to study certain aspects of [Natural Killer/Activated Natural

Killer] cell biology." Gong et al., 658; see also Gong et al., Abstract; see also Klingemann Decl., ¶ 22. Gong et al. also partially characterized the phenotype of NK-92 cells. Gong et al., 654. The NK-92 cell line was established from peripheral blood mononuclear cells of a fifty-year-old male patient who was diagnosed with an aggressive LGL lymphoma in 1992. Klingemann Decl., ¶ 21; '955 Appn., 14:4-5. While Gong et al. provide data that suggest that NK-92 cells kill K562 and Daudi cells in a chromium release assay (see Gong et al., 654 and Fig. 4), all experiments were performed *in vitro*. There is absolutely no teaching or suggestion in Gong et al. of a method of treating a pathology *in vivo* in a mammal comprising the step of administering to the mammal a medium comprising NK-92 cells, as in Applicant's independent claim 20.

The Examiner incorrectly states that "Gong et al. teach use of NK-92 cells to lyse leukemic tumor cells." Final Office Action, ¶ 8. Rather, Gong et al. teach that NK-92 cells demonstrated cytotoxicity against two human leukemic cell lines. Gong et al. simply do not teach that NK-92 cells are capable of lysing various tumor cells, including other leukemic tumor cells, of different origin or type. Klingemann Decl., ¶ 24a. As such, there is simply no teaching, suggestion, or motivation in Gong et al. that would lead one skilled in the art to use the NK-92 cell line *in vivo* to lyse tumor cells or as a cancer treatment, much less successfully reduce such a use to practice as a method of treating mammals. Id., ¶ 24c. Simply because a cell line is developed or established in the laboratory does not mean that there is an expectation of success for utilizing that cell line in a clinical setting. Certainly that was not the expectation with the NK-92 cell line because even the inventor did not initially recognize the importance or utility of the NK-92 cells in a clinical setting. Id.

2. Disclosure of Santoli et al.

Santoli et al. teach genetically modified cytotoxic T lymphoblastic leukemia (T-ALL) 104 and 107 cell lines and uses of these cell lines to treat cancer, both *in vivo* and *ex vivo*. Santoli et al., Abstract, 10:30-60. NK-92 cells are not disclosed by Santoli et al., nor is the use of NK-92 cells described. In fact, Santoli et al. do not provide a teaching, suggestion, or guidance with respect to any cell line other than T-ALL cells. In particular, Santoli et al. do not consider, teach, suggest, or provide guidance to NK-92 cells.

The Examiner misconstrues the teaching of Santoli et al. when, citing to Column 10 of Santoli et al., he states that "Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease or in preclinical in vivo studies." Final Office Action, ¶ 8. Rather, contrary to the Examiner's position, Santoli et al.'s teaching is limited to the use of "this invention," (i.e., T-ALL cells), not to all lytic human derived cell lines. The Examiner's conclusion is simply overly broad.

3. Santoli et al. is not relevant to Applicant's claimed method or combinable with Gong et al. because Santoli et al. disclose T-ALL cells which are structurally and functionally distinct

As set forth in the table below, the NK-92 cell line taught in Gong et al. or claimed by Applicant in independent claim 20 is structurally and functionally distinct from Santoli et al.'s T-ALL cell lines. Klingemann Decl., ¶ 28. As emphasized in Dr. Klingemann's declaration, know-how with respect to one cell line cannot automatically be transferred or applied to another cell line, even when the cells actually are closely related (which is not the case with NK-92 cells and T-ALL cells), including with respect to culture conditions, requirements for growth factors such as IL-2, survival and signaling patterns following adoptive transfer, ability to migrate to

tumor sites, sensitivity to chemotherapeutic agents, response to staining with vital dyes, ability to maintain their cytotoxic activity following radiation, and susceptibility to gene transfer. Id., ¶ 27. Furthermore, the know-how required to use a specific cell line as a method of treatment cannot automatically be transferred or applied to another cell line and is dependent on the distinguishing characteristics of each cell line. Id. Simply because one cell line has a specific utility does not mean that other closely related cell lines will have the same utility. Id. Each must be proven independently and the specific conditions necessary for successful results, including treatment, determined. Id.

Comparison of NK-92 cells to T-ALL cells

NK-92 Cells	T-ALL Cells
Derived from patient with aggressive LGL lymphoma	Derived from patient with T lymphoblastic leukemia
Originate from natural killer cells	Originate from T-cells
Do not require antibody stimulation in culture	Require antibody stimulation in culture
Maintain cytotoxicity and function after irradiation	Lose some cytotoxicity after irradiation
Have higher cytotoxicity than T-ALL cells	Have lower cytotoxicity than NK-92 cells

a. NK-92 cells and T-ALL cells were derived from different disease categories

The T-ALL cell line was derived from a patient with T lymphoblastic leukemia (T-ALL) (Santoli et al., 2:41-43), whereas the NK-92 cell line was derived from a patient with an aggressive LGL lymphoma. Klingemann Decl., ¶ 21. Leukemia and lymphoma are in different

disease categories and the cells derived therefrom are different cell lineages. Klingemann Decl., ¶ 28a. As such, the cell lines each have unique characteristics in culture and in undergoing proliferation. Id. As the inventor of the NK-92 cell line noted in his declaration, one skilled in the art would therefore assume that these two cell lines are different and that conclusions with respect to one of the cell lines cannot be drawn to the other cell line. Id.

b. NK-92 cells and T-ALL cells have different origins

T-ALL cells are of T-cell origin, are CD3-positive (a specific T-cell marker), CD8-positive, rearrange and express the T-cell receptor, are TCRαβ-positive, and are characterized by specific chromosomal translocations. *See* Santoli et al., 1:68, 2:14, and 4:27; *see also* Klingemann Decl., ¶ 28b. In addition, T-ALL cells lack natural cytotoxicity receptors such as NK-44 receptors that are found on NK-92 cells. In contrast, the NK-92 cell line is derived specifically from natural killer cells, making it a true NK cell line. Klingemann Decl., ¶ 28b. NK-92 cells are CD3-negative, CD8-negative, do not express or rearrange the T-cell receptor complex (TCR), and have different chromosomal rearrangements than T-ALL cells. Gong et al., 657-658; Klingemann Decl., ¶ 28b. As such, one cannot infer the behaviors, transfectability, or cytotoxic mechanisms of NK-92 cells from those of T-ALL cells because the cells have different phenotypes. Klingemann Decl., ¶ 28b.

c. NK-92 cells and T-ALL cells have different culture requirements

The culture for NK-92 cells is different from the culture for T-ALL cells. *See* Klingemann Decl., ¶ 28c. While T-ALL cells require antibody stimulation with CD2 or CD3 (a specific T cell marker) antigens to express IFN-γ, TNF-α, and GM-CSF (Santoli et al., 2:18, 47),

NK-92 cells do not require such antibody stimulation, but rather release these cytokines in response to stimulation by IL-2. Gong et al., 654; *see also* Klingemann Decl., ¶ 28d. Specifically, when NK-92 cells are cultured in α-minimum essential medium (α-MEM), the American Type Culture Collection (ATCC; Manassas, VA) recommends the media be supplemented with, among other things, 0.2 mM inositol, 0.1 mM 2-mercaptoethanol, 0.02 mM folic acid, 100-200 U/ml recombinant IL-2 (otherwise the cells die after 72 hours), and most surprisingly, a large proportion (25%) of two sera: 12.5% horse serum and 12.5% fetal bovine serum (FBS). In earlier passages, hydrocortisone is necessary. The cell density in culture is critical, and must be regularly checked and regulated by medium changes. The medium formulation, IL-2 concentration, serum concentration and cell density must be carefully regulated throughout the culture period. The culture of these cells are in stark contrast to other well-established cell lines (or even hybridomas), such as Madin-Darby Canine Kidney (MDCK) cells, which can thrive in simple MEM with 5% (FBS) and 2mM L-glutamine, 10mM N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), and sub-culturing once or twice a week.[2]

d. <u>NK-92 cells are more stable than T-ALL cells</u>

Additionally, NK-92 cells are more stable than T-ALL 104 cells. Tam et al. (Hum. Gene Ther., 10:1359-1373, 1999) have shown that NK-92 (both wild-type and transfected cells) cells require > 500 Gy to suppress proliferation, while Santoli et al., Cancer Res., 56:3021-3029, July 1996, reported that T-ALL 104 cells require 40 Gy irradiation to suppress proliferation.

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[2] Culture recommendations from the American Type Culture Collection (ATCC) for NK-92 cells are attached hereto.

Additionally, NK-92 cells maintain cytotoxicity and function even after irradiation, while T-ALL cells lose some cytotoxicity when irradiated. Klingemann Decl., ¶ 28e.

It has been reported that the standard treatment protocol for clinical trial in dogs required that the dogs be immunosuppressed using CsA, an immunosuppressive drug, starting the day before T-ALL 104 injections began and continuing through the first two weeks of T-ALL 104 injections. Santoli et al., Cancer Res., 56:3021-3029, July 1996. In contrast, NK-92 cells do not require supplemental immunosuppression. Klingemann Decl., ¶ 28f.

e. NK-92 cells have higher cytotoxic activity than T-ALL cells

Notably, comparative studies of NK-92 cells and T-ALL 104 cells further demonstrate that these cell lines are functionally quite different, with NK-92 cells having significantly higher cytotoxic activity than T-ALL 104 cells. For example, many hematological cancers are susceptible to killing by NK-92 cells, whereas these cancers are mostly resistant to lysis by T-ALL 104 cells. Klingemann Decl., ¶ 31. In fact, data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than T-ALL 104 cells or YT cells. See '955 Application, 35:11-36:20; 39:3-22; 44:11-28; Tables 5 and 6, Fig. 9. Even the inventor of the NK-92 cell line has indicated that the results demonstrating the superiority of the NK-92 cell line were surprising. Klingemann Decl., ¶ 33.

f. Summary

For at least these reasons, NK-92 cells are structurally and functionally different from the T-ALL cells disclosed by Santoli et al. One skilled in the art would therefore assume that conclusions with respect to one of these cell lines cannot be drawn to the other cell line. Klingemann Decl., ¶ 28a.

4. Applicant's method of treating a pathology as set forth in claim 20 is patentable over Gong et al. in view of Santoli et al. because Gong et al. merely established the NK-92 cell line and its phenotype while Santoli et al. is only relevant to T-ALL cells

Applicant disagrees with the Examiner's rejection of independent claim 20 for obviousness over Gong et al. in view of Santoli et al. because the combination of references fails to teach or suggest each and every element of Applicant's claimed method of treating a pathology *in vivo* by administering to the mammal NK-92 cells.

The Examiner has the burden pursuant to 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. In re Piasecki, 745 F.2d 1468 (Fed. Cir. 1984). To establish a *prima facie* case of obviousness, the Examiner must show: (i) a suggestion or motivation in the prior art, either from the references themselves or from generally available knowledge, for a person skilled in the art to choose the prior art reference or to combine the teachings of the references; (ii) a reasonable expectation of success; and (iii) that the reference or combination of references teach or suggest all of the claim limitations. See M.P.E.P. §§ 2141-2142; see also KSR Int'l Co. v. Teleflex, Inc., 127 S. Ct. 1727, 1741 (2007) (refusing to reject the use of teaching, suggestion, or motivation as a factor in the obviousness analysis because most inventions rely upon building blocks "long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known"). One court has noted that "[t]he KSR opinion only focused on the Federal Circuit's strict use of the [Teaching, Suggestion, Motivation] test in performing the obviousness analysis; it did not mention or affect the requirement that each and every claim limitation be found present in the combination of the prior art references before the analysis proceeds." Abbott Labs. V. Sandoz, Inc. 2007 U.S. Dist. Lexis 38216, *11 (N.D. Ill.

2007). Thus, KSR does not affect the Federal Circuit's holding that it is improper for the Examiner to use the applicant's invention as a blueprint to hunt through the prior art for the claimed elements and then combine them as claimed. See, e.g., In re Zurko, 111 F.3d 887 (Fed. Cir. 1997).

The Examiner has failed to meet his burden. Leaving aside the fact that Gong et al. limit their disclosure to establishing the NK-92 cell line, the differences between the NK-92 cells and T-ALL cells known at the time of filing Applicant's claimed method were so great that it was very unlikely that one skilled in the art would have found T-ALL cells to be any teaching with respect to NK-92 cells. The necessary nexus between the NK-92 cells taught by Gong et al. and an *in vivo* treatment of a pathology that would have led one skilled in the art to look to the teachings of Santoli et al. is missing. Simply because a cell line is developed or established in the laboratory does not mean that there is an expectation of success for utilizing that cell line in a clinical setting. Even the inventor of the NK-92 cell line did not initially recognize the importance or utility of the NK-92 cells in a clinical setting. Klingemann Decl., ¶ 24c. Additionally, the mere disclosure of NK-92 cells by Gong et al. is simply insufficient to obviate Applicant's claimed method and the Examiner's attempt to overcome the deficiencies of Gong et al. with the teachings of Santoli et al. is unfounded for a number of reasons, as detailed below.

First, any teaching, suggestion, or incentive in the prior art must not only motivate the skilled artisan to combine the teachings or suggestions, but must do so with a reasonable expectation of success. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art. In re Vaeck, 947 F.2d 488; M.P.E.P. § 2143.03. There is simply no such teaching, suggestion, or motivation in Gong et al.

to look to Santoli et al., let alone a reasonable expectation of success in combining those teachings. As set forth in detail above, the NK-92 cells disclosed by Gong et al. are phenotypically and functionally different from the T-ALL cells disclosed by Santoli et al. Because of these significant phenotypic and functional differences, there was simply no reason apparent to one skilled in the art at the time that Applicant's claimed method was filed to look to Santoli et al.'s teaching of T-ALL cells for any teaching with respect to a method of treating a pathology *in vivo* in a mammal by administering NK-92 cells, as is claimed by Applicant. Klingemann Decl., ¶ 29. Because of the significant and distinctive differences between these cell lines, the applicability and necessary requirements to use one of these cell lines as a method of treating *in vivo* is not applicable to the other, or to any other cell line for that matter. Id. Instead, the usefulness and necessary requirements for each would have to be characterized independently. Id. If one skilled in the art would have combined the teachings of Gong et al. and Santoli et al., the skilled artisan most certainly would not have had a reasonable expectation of success. Id., ¶ 30. In fact, the inventor of the NK-92 cell line has noted that application of the teachings of Santoli et al. to the NK-92 cells disclosed in Gong et al. would not have led to successful results because of the unique characteristics and requirements of the NK-92 cells. Id. Even with impermissible hindsight, one could not combine the teachings of Gong et al. and Santoli et al. to end up with Applicant's claimed method of treating a pathology *in vivo* by administering NK-92 cells because Applicant's NK-92 cell line is phenotypically and functionally different from Santoli et al.'s T-ALL cells.

Second, successful results and evidence of discovery further establish the patentability of Applicant's claimed method of treating a pathology *in vivo*. "[O]bjective evidence such as

commercial success, failure of others, long-felt need, and unexpected results must be considered before a conclusion on obviousness is reached." Minnesota Mining & Manufacturing Co. v. Johnson & Johnson Orthopedics, Inc., 976 F.2d 1559, 1573 (Fed. Cir. 1992) (noting the importance of secondary considerations in the obviousness analysis), citing Hybritech Inc. v. Monoclonal Antibodies, Inc., 802, F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986). Recent clinical trial studies demonstrated the "feasibility of large-scale expansion and safety of administering NK-92 cells as allogeneic cellular immunotherapy in advanced cancer patients and serves as a platform for future study of this novel natural killer (NK)-cell based therapy." Cytotherapy 10(6): 625-632, 2008. The methods used were tailored to NK-92 cells, which are very different from the methods tailored to T-ALL cells. Klingemann Decl., ¶ 35.

The Examiner alleges that:

> Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease whilst Gong et al. disclose that NK-92 cells are a lytic human derived cell line. In addition, as per the specification, page 2, last paragraph, use of NK cells and LAK cells to treat cancer in vivo was already known in the art. Gong et al. disclose that the NK-92 cell line displays characteristics of NK cells (see abstract), wherein use of NK cells to treat cancer in vivo was already known in the art.

Final Office Action, ¶ 8. The Examiner's conclusions are overly broad and misrepresent the disclosures of Santoli et al., Gong et al., and Applicant. Santoli et al. do not teach that all lytic human derived cell lines can be used *in vivo* to treat disease. Rather, Santoli et al. teach that T-ALL cells can be used in cancer therapy. See Santoli et al., 1:11-13. One skilled in the art would not extend such a limited teaching with respect to one cell line to be a teaching with respect to any other cell line. Klingemann Decl., ¶ 29. As discussed in detail above, there are significant phenotypic and functional differences between NK-92 cells and T-ALL cells, thereby

eliminating any reason for one skilled in the art at the time the claimed method was developed to look to Santoli et al.'s teaching of T-ALL cells to arrive at a method of treating a pathology *in vivo* in a mammal by administering NK-92 cells. Id., ¶ 29.

While the Examiner relies on Applicant's disclosure in the Specification (2:24-26) that "NK cells and LAK [lymphokine activated killer] cells have been used in both *ex vivo* therapy and *in vivo* treatment in patients with advanced cancer" to support his obviousness rejection, the Examiner fails to consider that NK cells and LAK cells are quite different from the claimed NK-92 cells and that Applicant's disclosure actually details the limitations of using NK and LAK cells *ex vivo* and *in vivo*. See '955 Application, 4:4-23. Applicant recognizes that "[t]here thus remains a need for a method of treating a pathology related to cancer or a viral infection with a natural killer cell line that maintains viability and therapeutic effectiveness against a variety of tumor classes." See '955 Application, 4:24-26. The Examiner has failed to recognize or consider that Applicant's claimed method, as set forth in claim 20, meets this need. See '955 Application, 5:4-5. While it was known in the art to use NK and LAK cells to treat a pathology, it was not known to use NK-92 cells for such a purpose until Applicant's claimed method was discovered. Gong et al.'s recognition in the Abstract that the novel NK-92 cell line "displays characteristics of activated NK-cells and could be a valuable tool to study their biology" does not impact the patentability of Applicant's claimed method because, at that time, there was absolutely no recognition that the NK-92 cells could be used *in vivo* as a method of treating, nor was there a motivation to look to Santoli et al. for such a teaching. Klingemann Decl., ¶ 29.

The Examiner goes on to support his rejection pursuant to 35 U.S.C. § 103(a) on the grounds that "in the post KSR Int'l Co. v. Teleflex Inc. universe, motivation per se is not even

required in a rejection under 35 U.S.C. § 103." Final Office Action, ¶ 8. Quoting KSR Int'l Co. v. Teleflex Inc., 550 U.S. m. 2007 WL 1237837 at 13 (2007), the Examiner states "if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill." Notably, the Examiner has acknowledged that "the two types of cells differ in phenotype" but has still concluded that "both the cells described by Santoli et al. and NK-92 are lytic human derived cell lines that can lyse various tumor cells." Final Office Action, ¶ 8. This conclusion is inaccurate because Gong et al. do not teach that NK-92 cells are capable of lysing various tumor cells of different origin or type. Klingemann Decl. ¶ 24. Instead, Gong et al. teach that NK-92 cells demonstrated cytotoxicity against two human leukemic cell lines in studies developed to characterize the newly isolated cell line. Id. Further, given that one skilled in the art would appreciate the significant phenotypic and functional differences between NK-92 cells and T-ALL cells, there would not have been any reason apparent to one skilled in the art at the time the claimed method was developed to look to Santoli et al.'s teaching of T-ALL cells to arrive at a method of treating a pathology *in vivo* in a mammal by administering NK-92 cells. Id., ¶¶ 27, 29. What the Examiner fails to appreciate is that Santoli et al. only teach methods applicable to T-ALL cells and do not provide guidance as to any other cell lines, while Gong et al. identify and partially characterize NK-92 cells which, at the time, was a new cell line. As discussed above, the inventor of the NK-92 cell line has noted that these two cell lines are from different cell lineages derived from different disease categories, leukemia and lymphoma. Id., ¶ 28. The T-ALL cell lines were derived from a patient with ALL, whereas the NK-92 cell line was derived from a patient with an aggressive LGL lymphoma. Id.

The actual application of a method for treating a pathology *in vivo* in a mammal by administering NK-92 cells would not have been obvious to a person of ordinary skill in the art based on the methods and teachings disclosed in Santoli et al. Id., ¶¶ 29, 30. The phenotypic and functional differences between the cells inherently prevent the know-how from one to be automatically transferred to the other, especially with any expectation of success. Id. Thus, contrary to the Examiner's conclusion, because Gong et al. do not teach a method of treating a pathology *in vivo*, it could not be obvious to use Gong et al. to arrive at, let alone improve, another technique.

The Examiner also asserts that "there is no teaching in Gong et al. that NK-92 cells are unacceptable for in vivo use." Final Office Action, ¶ 8. That notation, however, is irrelevant. It is the teaching of the reference that is relevant to an obviousness analysis, not what the reference does not teach. See, e.g., M.P.E.P. § 2143.01, citing KSR Int'l v. Teleflex Inc., 127 S.Ct. 1727, 1740-1741 (2007) (stating that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness"). Gong et al. do not teach or suggest that the NK-92 cells disclosed therein could be used *in vivo* to lyse tumor cells. Klingemann Decl., ¶ 24. This together with the fact that Santoli et al.'s teaching is limited to T-ALL cells renders the Examiner's combination of Gong et al. and Santoli et al. unsubstantiated.

The Examiner cites to M.P.E.P. § 2121, stating that "[w]hen the reference relied on expressly anticipates or makes obvious all of the elements of the claimed invention, the reference is presumed to be operable. Once such a reference is found, the burden is on the applicant to provide facts rebutting the presumption of operability." Final Office Action, ¶ 8. For the reasons set forth above, the Examiner has not established a *prima facie* case of obviousness.

Accordingly, the burden has not moved to Applicant to rebut the presumption of operability. However, even if the burden has moved to Applicant, the combination of Gong et al. and Santoli et al. would not have led to successful results because of the unique characteristics and requirements of these cells. Klingemann Decl., ¶ 30.

The Examiner also states that

> obviousness requires only a reasonable expectation of success. Regarding the Klingemann declaration, Santoli et al. teach that there is a need for cytotoxic cell lines which could be used to treat cancer. In view of the high level of skill in the art (Ph.D. or MD, with extensive research training) it would have been obvious to a routineer that other cytotoxic cell lines could be potentially used as per Santoli et al. In addition, the use of NK cells to treat cancer in vivo was already known in the art whilst Gong et al. disclose that the NK-92 cell line displays characteristics of NK cells.

Final Office Action, ¶ 8. As discussed above, there was not a reasonable expectation of success. As emphasized in the declaration of the inventor of the NK-92 cell line, the significant phenotypic and functional differences between NK-92 cells and T-ALL cells rendered the use of one of these cell lines as a method of treating *in vivo* inapplicable to the other, or to any other cell line for that matter, thereby precluding any expectation of success. Klingemann Decl., ¶¶ 29, 30. Additional comparative studies of NK-92 cells and TALL-104 cells further demonstrate that these cell lines are functionally quite different, with NK-92 cells having significantly higher cytotoxic activity than TALL-104 cells. Id., ¶ 31. For example, many hematological cancers are susceptible to killing by NK-92 cells, whereas these cancers are mostly resistant to lysis by TALL-104 cells. Id. In fact, data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than TALL-104 cells or YT cells. Id., ¶ 32. As further evidence of non-obviousness, the methods developed and being used in the clinic are very different for the two

cell lines. Reliance on the teachings of Santoli et al. would not have led to successful use of the NK-92 cells in a clinical setting. See, e.g., Id., ¶ 35.

With respect to Applicant's arguments that NK-92 cells and T-ALL cells are distinct cell lines, the Examiner alleges that Tam et al. state that "[a]n alternative is to use established cytotoxic NK tumor cell lines, which would give access to large numbers of effector cells. This concept has been proved by Cesano et al. (1997), who showed that an NK-like cell, TALL-104 was effective in treating a variety of malignancies in dogs." Final Office Action, ¶ 8. The Examiner continues: "contrary to the comments in the Klingemann declaration, Tam et al. disclose that TALL-104 is an NK-like cell line which is similar enough to NK cells that findings using TALL-104 cells can be extrapolated to NK cell lines." Final Office Action, ¶ 8. In fact, as set forth in Dr. Klingemann's declaration, Tam et al. actually demonstrate that NK-92 cells are more stable than T-ALL 104 cells. Klingemann Decl., ¶ 28e. Specifically, Tam et al. demonstrate that NK-92 cells and T-ALL cells are phenotypically distinct because Tam et al. showed that NK-92 cells require >500 Gy to suppress proliferation, while others have reported that T-ALL 104 cells require 40 Gy irradiation to suppress proliferation. See Santoli et al., Cancer Res., 56: 3021-3029, July 1996. Additionally, NK-92 cells maintain cytotoxicity and function even after irradiation, while T-ALL cells lose some cytotoxicity when irradiated. Klingemann Decl., ¶ 28e. NK-92 cells do not require supplemental immunosuppression. Id., ¶ 28f. Accordingly, T-ALL cells are immunogenic while NK-92 cells are not. Id.

The Examiner also alleges that "Klingemann et al. (1996) also disclose that NK-92 and TALL-104 cells have similar lytic properties." Final Office Action, ¶ 8. In fact, that is a misrepresentation of Klingemann et al. That reference actually acknowledges that "[a]

comparative study of the cytotoxic activity of the TALL-104 and the NK-92 cells has suggested, however, that NK-92 cells display a higher level of cytotoxicity than TALL-104 cells against leukemic and lymphoma targets and also lyse a broader spectrum of leukemic target cells including primary leukemias derived from patients." Klingemann et al., Biol. Blood Marrow Transplant., 2:68-75, 73 (1996). As set forth in detail above, data actually have demonstrated that NK-92 cells are, in fact, superior to T-ALL cells. See Klingemann Decl., ¶¶ 31-33.

The Examiner alleges that "there is no evidence of record that in vivo treatment with NK-92 cells is superior to in vivo treatment with TALL-104 cells." Final Office Action, ¶ 8. The Examiner is incorrect. See, e.g., Klingemann Decl., ¶¶ 31-33 (stating that "data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than TALL-104 cells or YT cells"). In fact, data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than T-ALL 104 cells or YT cells. See '955 Application, 35:11-36:20; 39:3-22; 44:11-28; Tables 5 and 6, Fig. 9.

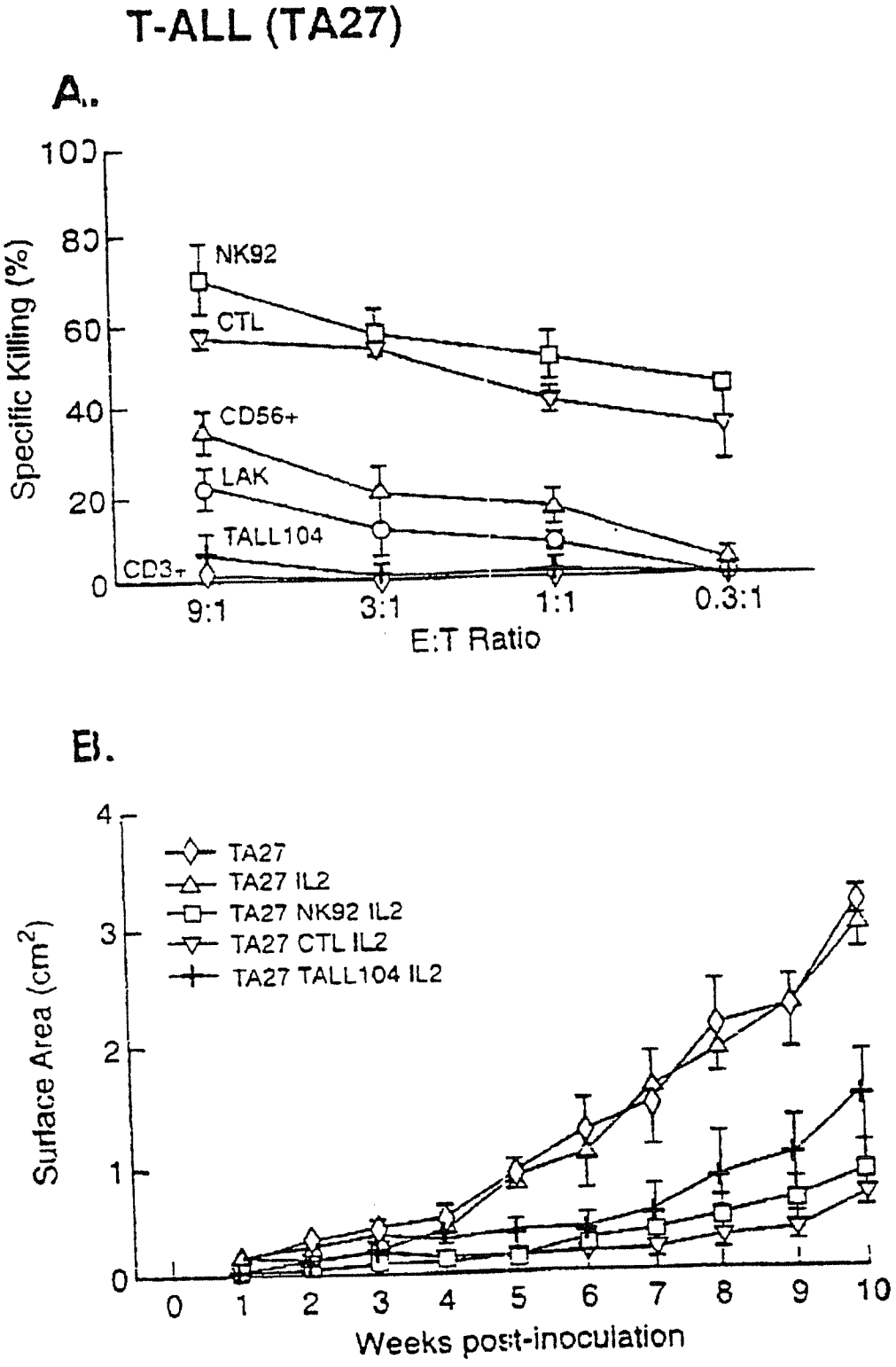
T-ALL (TA27)
A.
Specific Killing (%)
100
80
60
40
20
0
NK92
CTL
CD56+
LAK
TALL104
CD3+
9:1
3:1
1:1
0.3:1
E:T Ratio
B.
Surface Area (cm²)
4
3
2
1
0
TA27
TA27 IL2
TA27 NK92 IL2
TA27 CTL IL2
TA27 TALL104 IL2
0 1 2 3 4 5 6 7 8 9 10
Weeks post-inoculation

FIGURE 9

'955 Application, Fig. 9. These results demonstrate that the NK-92 cell line and the T-ALL 104 cell line are not even comparable. Klingemann Decl., ¶ 32. In fact, the inventor of the NK-92 cell line found these results to be surprising. Id., ¶ 33.

In fact, results recently published by the inventor are promising and encourage continued development of the use of NK-92 cells as a method of treatment. Klingemann Decl., ¶ 35. This study confirmed the feasibility of large-scale expansion and safety of administering *ex vivo* expanded NK-92 cells as allogeneic cellular immunotherapy in patients with refractory renal cell cancer and melanoma. See Arai et al., Cytotherapy, 10(6): 625-632 (2008) (a copy of which is attached hereto).

For at least the reasons discussed above, Gong et al. does not teach or suggest each and every element of Applicant's claimed method of treating a pathology. Because Gong et al. fail to teach or suggest each and every one of Applicant's claimed elements, Santoli et al.'s alleged teaching with respect to *in vivo* treatment by T-ALL cells becomes moot. The addition of Santoli et al. to Gong et al. does not ameliorate the deficiencies of Gong et al. as an obviating reference. Therefore, the rejection of claim 20 cannot stand.

5. Applicant's methods of treating a pathology as set forth in dependent claims 22, 26, 27, and 30 are also patentable over Gong et al. in view of Santoli et al.

The Examiner alleges that dependent claim 22, which depends from claim 20, is also obviated by the combination of Gong et al. in view of Santoli et al. Applicant disagrees with the Examiner's rejection of dependent claim 22 because dependent claim 22 also requires that the pathology is a cancer. Claim 22 is allowable by virtue of its dependency on claim 20, which is allowable for at least the reasons set forth above. *See* M.P.E.P. § 2143.03 (stating that "[i]f an

independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious"); see also In re Fine, 837 F.2d 1071, 5 USPQ2d 1506 (Fed. Cir. 1988). Accordingly, any teaching with respect to the pathology being a cancer is rendered moot because Gong et al. in view of Santoli et al. fail to teach or suggest each and every element of Applicant's claimed method treating a pathology for at least the reasons set forth above. The Examiner's rejection of claim 22 cannot stand.

The Examiner also alleges that dependent claim 26, which depends from claim 20, is also obviated by the combination of Gong et al. in view of Santoli et al. Applicant disagrees with the Examiner's rejection of dependent claim 26 because dependent claim 26 also requires that the cells be administered to a human intravenously. Claim 26 is allowable by virtue of its dependency on claim 20, which is allowable for at least the reasons set forth above. *See* M.P.E.P. § 2143.03 (stating that "[i]f an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious"); see also In re Fine, 837 F.2d 1071, 5 USPQ2d 1506 (Fed. Cir. 1988). Accordingly, any teaching with respect to the route of administration of the cells to the mammal being intravenous and the mammal being human is rendered moot because Gong et al. in view of Santoli et al. fail to teach or suggest each and every element of Applicant's claimed method treating a pathology for at least the reasons set forth above. The Examiner's rejection of claim 26 cannot stand.

The Examiner also alleges that dependent claim 27, which depends from claim 20, is also obviated by the combination of Gong et al. in view of Santoli et al. Applicant disagrees with the Examiner's rejection of dependent claim 27 because dependent claim 27 also comprises the step of administering to the mammal a cytokine that promotes the growth of NK-92 cells. Claim 27

is allowable by virtue of its dependency on claim 20, which is allowable for at least the reasons set forth above. *See* M.P.E.P. § 2143.03 (stating that "[i]f an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious"); see also In re Fine, 837 F.2d 1071, 5 USPQ2d 1506 (Fed. Cir. 1988). Santoli et al. do not disclose a method of treating comprising the step of administering to the mammal a cytokine that promotes the growth of NK-92 cells. Rather, Santoli et al. disclose "incorporating into the cell line a selected lymphokine gene." Santoli et al., 7:29-34. Thus, Santoli et al.'s teaching cannot obviate Applicant's dependent claim 27 because the combination of Gong et al. in view of Santoli et al. fail to teach or suggest Applicant's claimed method. The Examiner's rejection of claim 27 cannot stand.

The Examiner also alleges that dependent claim 30, which depends directly from claim 22 (and indirectly from claim 20), is also obviated by the combination of Gong et al. in view of Santoli et al. Applicant disagrees with the Examiner's rejection of dependent claim 30 because dependent claim 30 also requires that the cancer be a solid tumor. Claim 30 is allowable by virtue of its dependency on claim 20, which is allowable for at least the reasons set forth above. *See* M.P.E.P. § 2143.03 (stating that "[i]f an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious"); see also In re Fine, 837 F.2d 1071, 5 USPQ2d 1506 (Fed. Cir. 1988). Accordingly, any teaching with respect to the cancer being a solid tumor is rendered moot because Gong et al. in view of Santoli et al. fail to teach or suggest each and every element of Applicant's claimed method treating a pathology for at least the reasons set forth above. The Examiner's rejection of claim 30 cannot stand.

D. Conclusion

For at least the reasons set forth herein, Applicant respectfully requests that the Board reverse the Examiner's final rejection and allow all claims because the Examiner has failed to show or establish how Gong et al. in combination with Santoli et al. obviates Applicant's claimed invention. In accordance with the above remarks, claims 20, 22, 26, 27, and 30 are patentable over the cited references and allowance of same is hereby respectfully requested.

Applicant does not believe that a fee is due. However, if the Commissioner determines that a fee is required, the Commissioner is authorized to charge any required fee to Deposit Account No. 03-2026.

Respectfully submitted,

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VIII. CLAIMS APPENDIX

The following claims are the claims on appeal as presently amended:

1. (Withdrawn) A method of purging cells related to a pathology from a biological sample, said method comprising (i) obtaining a biological sample from a mammal, wherein the biological sample is suspected of containing cells related to the pathology, and (ii) contacting the biological sample with a medium comprising NK-92 or modified NK-92 natural killer cells, wherein the modified NK-92 cells have been modified by a physical treatment or by transfection with a vector; whereby the natural killer cells purge cells related to the pathology from the sample.

2. (Withdrawn) The method described in claim 1 wherein the pathology is a cancer.

3. (Withdrawn) The method described in claim 1 wherein the pathology is an infection by a pathogenic virus.

4. (Withdrawn) The method described in claim 3 wherein the pathogenic virus is human immunodeficiency virus, Epstein-Barr virus, crytomegalovirus, or herpes virus.

5. (Withdrawn) The method described in claim 1 wherein the biological sample is human blood or bone marrow.

6. (Withdrawn) The method described in claim 1 wherein the natural killer cell is immobilized on a support.

7. (Withdrawn) The method described in claim 1 wherein the modified NK-92 cells have been modified by a physical treatment that renders them non-proliferative, said treatment not significantly diminishing their cytotoxicity, by treatment that inhibits express of HLA antigens on the NK-92 cell surface, by transfectoin with a vector, or by any combination thereof.

8. (Withdrawn) The method described in claim 7 wherein the cells have been transfected with a vector encoding a cytokine that promotes the growth of the cells, a vector encoding a protein that is responsive to an agent, a vector encoding a cance cell receptor molecule, or with any combination thereof.

9. (Withdrawn) The method described in claim 1 wherein the medium further comprises cytokine that promotes the growth of the cells.

10. (Withdrawn) A method of treating a pathology ex vivo in a mammal comprising the steps of:

(i) obtaining a biological sample from the mammal, wherein the sample is suspected of containing cells related to the pathology;

(ii) contacting the biological sample with a medium comprising NK-92 or modified NK-92 natural killer cells, wherein the modified NK-92 cells have been modified by a physical treatment or by transfection with a vector, whereby the cells related to the pathology in the sample are selectively destroyed, thereby producing a purged sample; and

(iii) returning the purged sample to the mammal.

11. (Withdrawn) The method described in clam 10 wherein the pathology is a cancer.

12. (Withdrawn) The method described in claim 11 wherein the cancer is a leukemia, a lymphoma or a multiple myeloma.

13. (Withdrawn) The method described in claim 10 wherein the pathology is an infection by a pathogenic virus.

14. (Withdrawn) The method described in claim 13 wherein the pathogenic virus is human immunodeficiency virus, Epstein-Barr virus, cytomegalovirus, or herpes virus.

15. (Withdrawn) The method described in claim 10 wherein the biological sample is blood or bone marrow and wherein the mammal is a human.

16. (Withdrawn) The method described in claim 10 wherein the natural killer cell is immobilized on a support.

17. (Withdrawn) The method described in claim 10 wherein the medium comprises modified NK-92 cells which have been modified by a physical treatment that renders them non-proliferative, said treatment not significantly diminishing their cytotoxicity, by treatment that inhibits expression of HLA antigens on the NK-92 cell surface, by transfection with a vector, or by any combination thereof.

18. (Withdrawn) The method described in claim 17 wherein the cells have been transfected with a vector encoding a cytokine that promotes the growth of the cells, a vector encoding a protein that is responsive to an agent, a vector encoding a cancer cell receptor molecule, or with any combination thereof.

19. (Withdrawn) The method of treating a cancer described in claim 10 wherein the medium further comprises a cytokine that promotes the growth of the cells.

20. (Previously presented) A method of treating a pathology *in vivo* in a mammal comprising the step of administering to the mammal a medium comprising NK-92 cells (available from American Type Culture Collection (ATCC) as Deposit No. CRL-2407).

21. (Withdrawn) The method described in claim 20 wherein the modified NK-92 cells have been transfected with a vector encoding a cytokine that promotes the growth of the cells, with a vector encodinga protein that is responsive to an agent, a vector encoding a cancer cell receptor molecule, or with any combination thereof.

22. (Previously presented) The method described in claim 20 wherein the pathology is a cancer.

23. (Withdrawn) The method of treating a pathology described in claim 31 wherein the cancer is a leukemia, a lymphoma or a multiple myeloma.

24. (Withdrawn) The method described in claim 20 wherein the pathology is an infecton by a pathogenic virus.

25. (Withdrawn) The method described in claim 24 wherein the pathogenic virus is human immunodeficiency virus, Epstein-Barr virus, cytomegalovirus, or herpes virus.

26. (Previously presented) The method of treating a pathology described in claim 20 wherein the route of administration of the cells to the mammal is intravenous and the mammal is human.

27. (Previously presented) The method of treating a pathology described in claim 20 further comprising the step of administering to said mammal a cytokine that promotes the growth of said NK-92 cells.

28. (Withdrawn) The method of treating a pathology decribed in claim 26 wherein the NK-92 is modified by transfection with a vector encoding a protein that is responsive to an agent such that when the agent is taken up by the cell, the cell is inactivated, and wherein the method further comprises administering to the mammal, after a time sufficient for the natural killer cell to treat the cancer has elapsed, an amount of the agent effective to inactivate the cell.

29. (Withdrawn) The method of treating a pathology described in claim 28 wherein the agent is acyclovir or gancyclovir.

30. (Previously presented) The method of treating a pathology described in claim 22 wherein the cancer is a solid tumor.

31. (Withdrawn) The method of treating a pathology described in claim 22 wherein the cancer is a non-solid tumor of circulating cells.

IX. EVIDENCE APPENDIX

(1) Declaration of Hans Klingemann, M.D., Ph.D. Pursuant to 37 C.F.R. § 1.132, filed on October 15, 2008, in support of the Request for Continued Examination filed on October 15, 2008, in response to the Final Office Action mailed on April 15, 2008.

(2) Arai et al., Cytotherapy, 10(6): 625-632 (2008).

(3) Culture recommendations from the American Type Culture Collection (ATCC) for NK-92 cells.

X. RELATED PROCEEDINGS APPENDIX

(1) U.S. Patent Application No. 10/701,359, filed on November 4 2003, entitled "Methods of Treating Tumors Using Natural Killer Cell Lines," which is a divisional of the '955 Application, which is a continuation-in-part of U.S. Patent Application No. 09/403,910, filed on October 27, 1999, now abandoned, which is a national phase entry of PCT/US98/08672, filed on April 30, 1998 and which claims priority to U.S. Provisional Application No. 60/045,885, now expired, filed on April 30, 1997.

(2) U.S. Patent Application No. 10/456,237, filed on June 6, 2003, entitled "Interleukin-Secreting Natural Killer Cell Lines and Methods of Use," which is a divisional of the '955 Application, which is a continuation-in-part of U.S. Patent Application No. 09/403,910, filed on October 27, 1999, now abandoned, which is a national phase entry of PCT/US98/08672, filed on April 30, 1998 and which claims priority to U.S. Provisional Application No. 60/045,885, now expired, filed on April 30, 1997.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Hans Klingemann	)	
	)	
Serial No. 10/008,955	)	**NATURAL KILLER CELL LINES AND METHODS OF USE**
	)	
Filed: December 7, 2001	)	
	)	
Art Unit: 1644	)	
	)	
Patent Examiner: Ronald B. Schwadron	)	
	)	
Attorney Docket No. 06-129 PCT/US/CIP	)	
	)	
Confirmation No.: 5420	)	

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November 16, 2009

APPEAL BRIEF PURSUANT TO 37 C.F.R. § 41.37

Pursuant to 37 C.F.R. §§ 41.31 and 41.37, Applicant hereby submits the following brief to the U.S. Patent and Trademark Office ("PTO") Board of Appeals and Interferences ("the Board") in support of Applicant's appeal of the Examiner's decision in the final Office Action mailed on March 24, 2009, ("Final Office Action") to finally reject claims 20, 22, 26, 27, and 30. For at least the reasons set forth herein Applicant respectfully submits that the claims as currently presented are patentable and requests that the Board reverse the Examiner's final rejection thereof, remand this proceeding to the Examiner, and order the Examiner to issue a notice of allowance.

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TABLE OF AUTHORITIES

Cases

Statutes

Other Authorities

I. REAL PARTY IN INTEREST

The subject application has been assigned to ZelleRx Corporation who is the real party in interest.

II. RELATED APPEALS AND INTERFERENCES

The following cases are related to U.S. Patent Appn. No. 10/008,955 ("the '955 Application"), currently on appeal:

(1) U.S. Patent Application No. 10/701,359, filed on November 4 2003, entitled "Methods of Treating Tumors Using Natural Killer Cell Lines," which is a divisional of the '955 Application, which is a continuation-in-part of U.S. Patent Application No. 09/403,910, filed on October 27, 1999, now abandoned, which is a national phase entry of PCT/US98/08672, filed on April 30, 1998 and which claims priority to U.S. Provisional Application No. 60/045,885, now expired, filed on April 30, 1997.

(2) U.S. Patent Application No. 10/456,237, filed on June 6, 2003, entitled "Interleukin-Secreting Natural Killer Cell Lines and Methods of Use," which is a divisional of the '955 Application, which is a continuation-in-part of U.S. Patent Application No. 09/403,910, filed on October 27, 1999, now abandoned, which is a national phase entry of PCT/US98/08672, filed on April 30, 1998 and which claims priority to U.S. Provisional Application No. 60/045,885, now expired, filed on April 30, 1997.

III. STATUS OF CLAIMS

Claims 20, 22, 26, 27, and 30 are currently pending. Claims 1-19, 21, 23-25, 28, 29, and 31 are withdrawn from consideration. For convenience, the complete text of the claims is

attached hereto in the Claims Appendix in Section VIII hereto. Claims 20, 22, 26, 27, and 30 were finally rejected and are on appeal:

(a) Claims 20, 22, 26, 27, and 30 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 30-35, 46, 48, 50, and 53 of co-pending U.S. Appn. No. 10/701,359 ("the '359 Application"). Applicant indicated in the Request for Continued Examination filed on October 15, 2008 that a Terminal Disclaimer will be filed upon recognition of allowable subject matter.

(b) Claims 20, 22, 26, 27, and 30 are rejected pursuant to 35 U.S.C. § 103(a) as being unpatentable over Gong et al., *Leukemia* 8:652-658, 1994 ("Gong et al.") in view of U.S. Patent No. 5,272,082 to Santoli et al. ("Santoli et al.").

IV. STATUS OF AMENDMENTS

Applicant filed an amended claim set with the Request for Continued Examination filed on October 15, 2008[1]. Applicant has not filed any amendment subsequent to the Final Office Action mailed on March 24, 2009.

Applicant filed the Notice of Appeal on September 15, 2009.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Independent claim 20 is directed to a method of treating a pathology *in vivo*. See the '955 Application as originally filed, pg. 1, lines 8-10 (hereinafter, "the '955 Appn., __:__"); see also the '955 Appn. 4:24-26; 5:9-11; 8:9-26; 13:11-20; 15:29-16:5; 16:19-24; 23:24-24:2; 26:18-22; 35:12-25; 39:3-10; 39:19-22; 41:26-42:5; 43:22-29; 44:12-28;

46:24-27; Table 1; Table 5; Table 6; Figure 8; and Figures 11-13). The method is carried out in a mammal. See the '955 Appn., 8:10; 13:16; 18:23-26; 23:24-25; 41:26-30; and 44:1-46:27. Applicant developed a unique cell line identified as NK-92 and available from American Type Culture Collection (ATCC) as Deposit No. CRL-2407. The claimed method comprises the step of administering to the mammal a medium comprising NK-92 cells. See the '955 Appn., 1:10; 8:11; 13:17-18; 14:4-5; 16:11-25; 23:25; and 25:10-12.

Dependent claim 22, which depends from claim 20, is directed to a method wherein the pathology is a cancer. See the '955 Appn., 1:9; 4:25; 5:10; 13:11-13; 23:23-24:2).

Dependent claim 26, which depends from claim 20, is directed to a method wherein the cells are administered to the mammal intravenously. See the '955 Appn. 8:21-22; 23:26-27; 23:29-24:2). The mammal is a human. See the '955 Appn. 8:21-22.

Dependent claim 27, which depends from claim 21, further comprises the step of administering to the mammal a cytokine that promotes the growth of the NK-92 cells. See the '955 Appn., 8:21-24; 24:13-23).

Dependent claim 30, which depends from claim 22, is directed to a solid tumor cancer. See the '955 Appn., 23:29.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1) The rejection of claims 20, 22, 26, 27, and 30 pursuant to 35 U.S.C. § 103(a) as being unpatentable over Gong et al. in view of Santoli et al. is being appealed.

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[1] The Request for Continued Examination was refiled on January 15, 2009 in the Response to Notice of Non-Compliant Amendment to correct the status identifiers of claims 23 and 31 that were improperly labeled as

2) The rejection of claims 20, 22, 26, 27, and 30 on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 30-35, 46, 48, 50, and 53 of co-pending Application No. 10/701,359 is not being appealed. A terminal disclaimer will be submitted upon an indication of allowability of the pending claims.

3) The Examiner's refusal to enter the Substitute Specification on the grounds that it does not conform to 37 C.F.R. § 1.125(b), (c) because it allegedly contains new matter is not being appealed. Applicant will cancel the alleged new matter upon an indication of allowability of the pending claims.

4) The objection to the amendment filed on October 15, 2008 pursuant to 35 U.S.C. § 132(a) on the grounds that new matter is introduced into the disclosure is not being appealed. As set forth above, Applicant will cancel the new matter upon an indication of allowability of the pending claims.

VII. ARGUMENT

A. Introduction

This appeal is based on Applicant's belief that the Examiner has failed to establish a *prima facie* case of obviousness on which to base the current rejections of the claims pursuant to 35 U.S.C. § 103(a). The Examiner's rejection is erroneously premised on the combination of two references, namely Gong et al. in view of Santoli et al., despite the fact that the combination of references fails to teach or suggest Applicant's method of treating a pathology *in vivo* comprising the step of administering to the mammal a medium comprising NK-92 cells. Further support for the lack of obviousness is found in the Declaration of Hans Klingemann, M.D.,

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"withdrawn – previously presented" rather than "withdrawn."

Ph.D., pursuant to 37 C.F.R. § 1.132 (hereinafter, "Klingemann Decl."), the sole inventor of the NK-92 cells disclosed in the '955 Application and a skilled artisan in the fields of translational research, transplantation biology, and tumor immunology. Klingemann Decl., ¶ 14.

Gong et al. disclosed the NK-92 cell line, an immortal cell line originally obtained from peripheral mononuclear cells of a fifty-year-old male patient having non-Hodgkin's lymphoma. Klingemann Decl., ¶ 21; '955 Appn., 14:4-5. At the time that the NK-92 cell line was discovered, the inventor thought that the cell line provided a suitable model to study the biology of NK-cell and activated NK-92 cells. Klingemann Decl., ¶ 22. Gong et al. merely set out to characterize the NK-92 cell line for use as a research tool. Gong et al., Abstract.

Further research with the NK-92 cell line revealed surprising and unexpected results, and it is these further inventions that are claimed in the '955 Application. '955 Appn., 43:22-29. Of particular surprise was the finding that NK-92 cells have cytolytic activity *in vitro* and tumor-inhibiting activity *in vivo*. '955 Appn. 43:22-29. Specific data demonstrating the cytoxic activity of the NK-92 cells are set out in Tables 5 and 6 and Figure 9 of the '955 Application, reproduced below.

Table 5. Cytotoxicity of NK-92, T-ALL104 and YT Clone to Patient-Derived Leukemic Cells[a]

Patient	Disease Status	Blast (%) in Sample	Cytotoxic Sensitivity		
			NK-92	TALL-104	YT
AML					
1 M4□	Relapse	PB (66%)	++++++	+++++	-
2 (M1)	Relapse	PB (50%)	+++++	-	-
3 (M3)	Relapse	PB (50%)	+++ (++++)	+ (++++)	- (-)
4 (M4)	Refractory	PB (90%)	++ (++)	- (+)	- (-)
5 (M2)	New	BM (90%)	+++ (+++)	+ (+++)	ND
6 (M4)	New	BM (97%)	-	-	-
7 (M4)	New	PB (39%)	- (-)	- (++)	- (-)
8 (M3)	New	PB (55%)	- (++)	- (+++)	+ (-)
9 (M3)	New	BM (32%)	-	-	-
T-ALL					
1	Relapse	BM (98%)	++++++	-	-
2	Relapse	PB (85%)	++++++	- (-)	+++ (+++)
3	Relapse	PB (77%)	++++++	- (+)	- (-)
4	Relapse	PB (60%)	+++++	- (-)	+ (-)
5	New	BM (40%)	+++	-	-
6	New	BM (66%)	+++	-	-
B-Lineage-All					
1 ●	Relapse	BM (78%)	+++++	++++	-
2	New	BM (30%)	++++	ND	ND
3	Relapse	BM (75%)	+++ (++++)	+ (++++)	++ (++)
4	New	BM (97%)	++ (+++)	+ (+++)	- (-)
5	Relapse	BM (60%)	+ (+)	- (+)	- (-)
6	Relapse	BM (80%)	-	ND	ND
7	Relapse	PB (80%)	-	- (-)	-
8	New	BM (68%)	-	-	-
9	New	BM (33%)	-	- (+)	-

Patient	Disease Status	Blast (%) in Sample	Cytotoxic Sensitivity		
			NK-92	TALL-104	YT
10	Relapse	BM (87%)	-	- (++)	-
11	Relapse	BM (75%)	- (+++)	- (++++)	-
12	New	BM (30%)	-	-	ND
13	New	PB 90%)	- (+++)	- (+++)	ND
14	New	BM (81%)	-	-	ND
CML					
1	BC	PB (45%)	++++++	+++++	+++
2	AC	PB (22%)	++++++	++	-
3	BC	PB (93%)	+++++	+	-
4	CP	PB (15%)D	++++	+	-
5	CP	PB (8%)D	++ (++++)	ND	ND
6	CP	BM (12%)D	+ (+++)	+ (+)	ND
7	CP	BM (10%)D	+ (+++)	+ (++++)	ND
8	BC	PB (60%)	+	-	-
9	BC	BM (48%)	+	- (-)	-
10	CP	PB (21%)D	+ (++)	- (++++)	- (-)
11	CP	PB (11%)D	-	- (+++++)	- (-)

Notes and Abbreviations. a) Columns show results of chromium release assays at E:T = 9:1 after 4 h without parentheses, and (results after 18 h enclosed in parentheses); New: newly diagnosed; ND: none done; o: FAB classification; D:blast and promyelocyte; BM: bone marrow; PB: peripheral blood; I:B-ALL; BC: blast crisis; AC: accelerated phase; CP: chronic phase.

Table 6. Specific Lysis of Human Leukemia Cell Lines by Natural Killer Cell ClonesNK-92, TALL-104, and YT.

Target	Specific Lysis (%)								
	NK92			TALL-104			YT		
	Effector:Target Ratio								
	9:1	3:1	1:1	9:1	3:1	1:1	9:1	3:1	1:1
K562	94.1	91.2	82.1	88.5	85.2	72.5	34.2	28.2	18.4
HL60	87.9	75.3	79.6	43.0	16.0	6.9	2.1	1.1	1.5
KG1	64.6	53.8	43.7	2.7	0.5	0	0.1	0	0
NALM6	72.6	56.8	52.4	67.8	55.6	33.3	1.0	0.5	0
Raji	86.0	75.4	70.0	22.2	10.2	0.3	25.1	18.0	14.2
TALL-104	57.3	53.2	44.1	-	-	-	3.2	1.4	0.9
CEM/S	56.6	48.8	34.7	2.7	1.6	0.9	0.9	0.4	0.3
CEM/T	57.5	42.1	39.1	1.5	0.6	0.3	1.2	0.1	0.2

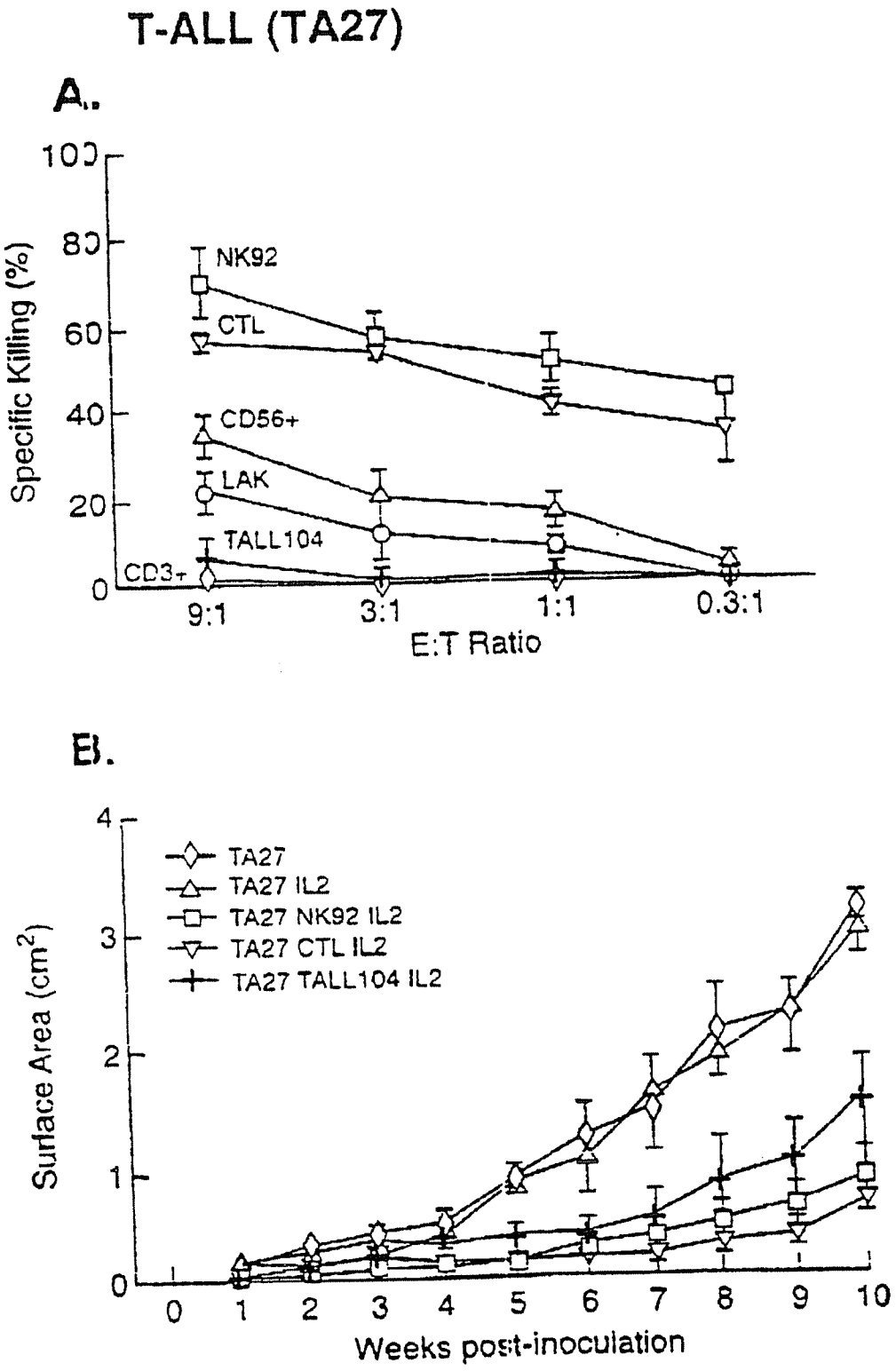
T-ALL (TA27)
A.
100
80
60
40
20
0
Specific Killing (%)
NK92
CTL
CD56+
LAK
TALL104
CD3+
9:1
3:1
1:1
0.3:1
E:T Ratio
B.
4
3
2
1
0
Surface Area (cm²)
TA27
TA27 IL2
TA27 NK92 IL2
TA27 CTL IL2
TA27 TALL104 IL2
0 1 2 3 4 5 6 7 8 9 10
Weeks post-inoculation

FIGURE 9

In contrast to the teachings of Gong et al., Santoli et al. teach "genetically modified cytotoxic T lymphoblastic leukemia cell lines (T-ALL), and uses of these cell lines in cancer therapy." Santoli et al., Abstract. There is absolutely no teaching or suggestion of Applicant's claimed method in either Gong et al. or Santoli et al., alone or in combination. As such, the Examiner's rejection of claim 20 and claims 22, 26, 27, and 30 depending therefrom is completely unsubstantiated. The rejection should be reversed.

B. Applicant's Claimed Method of Treating a Pathology *In Vivo*

Unlike anything shown in the combination of Gong et al. and Santoli et al., Applicant's independent claim 20 claims a method of treating a pathology *in vivo* by administering a medium comprising a particular line of NK-92 cells.

Dependent claim 22, which depends from claim 20, is for a method of treating a cancer.

Dependent claim 26, which depends from claim 20, provides that the NK-92 cells are administered to the mammal intravenously and that the mammal is a human.

Dependent claim 27, which dependents from claim 20, further comprises the step of administering to the mammal a cytokine that promotes the growth of the NK-92 cells.

Dependent claim 30, which depends from claim 22, limits the cancer to a solid tumor.

C. For the reasons explained herein, the Examiner cannot properly rely on the combination of Gong et al. and Santoli et al. to support a rejection of the claims pursuant to 35 U.S.C. § 103(a).

C. Independent claim 20 is not obvious over Gong et al. in view of Santoli et al. because claim 20 recites subject matter not shown or suggested by the cited prior art.

Pursuant to 35 U.S.C. § 103(a), the Examiner has finally rejected claims 20, 22, 26, 27, and 30 directed to a method of treating a pathology *in vivo* in a mammal comprising the step of administering to the mammal a medium comprising NK-92 cells (available from ATCC as Deposit No. CRL-2407) as being unpatentable over Gong et al. in view of Santoli et al.

Specifically, the Examiner alleges that

> Gong et al. teach use of NK-92 cells to lyse leukemic tumor cells. Gong et al. teach that said cells require IL-2 to function. Gong et al. does not in vivo use of NK-92 cells to treat cancer [sic]. Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease or in preclinical in vivo studies. Santoli et al. teach that said cells are injected iv wherein injection utilizes a syringe and wherein the injected NK-92 cells would be adjacent to leukemic cells in the blood. Santoli et al. disclose that the cells can be administered with the cytokine IL-2. Santoli et al. teach that said cells can be modified to bind solid tumors. It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have created the claimed invention because Gong et al. teach use of NK-92 cells to lyse tumor cells, while Santoli et al. teach in vivo use of cytotoxic cell lines. One of ordinary skill in the art would have been motivated to do so because Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease or in preclinical in vivo studies.

Final Office Action, ¶ 8. Applicant disagrees for at least the reasons set forth below.

1. Disclosure of Gong et al.

Gong et al. established the existence of the immortal cell line, NK-92, and set out to characterize the NK-92 cell line for use as a research tool, concluding that the NK-92 cell line "may provide a suitable model to study certain aspects of [Natural Killer/Activated Natural

Killer] cell biology." Gong et al., 658; see also Gong et al., Abstract; see also Klingemann Decl., ¶ 22. Gong et al. also partially characterized the phenotype of NK-92 cells. Gong et al., 654. The NK-92 cell line was established from peripheral blood mononuclear cells of a fifty-year-old male patient who was diagnosed with an aggressive LGL lymphoma in 1992. Klingemann Decl., ¶ 21; '955 Appn., 14:4-5. While Gong et al. provide data that suggest that NK-92 cells kill K562 and Daudi cells in a chromium release assay (see Gong et al., 654 and Fig. 4), all experiments were performed *in vitro*. There is absolutely no teaching or suggestion in Gong et al. of a method of treating a pathology *in vivo* in a mammal comprising the step of administering to the mammal a medium comprising NK-92 cells, as in Applicant's independent claim 20.

The Examiner incorrectly states that "Gong et al. teach use of NK-92 cells to lyse leukemic tumor cells." Final Office Action, ¶ 8. Rather, Gong et al. teach that NK-92 cells demonstrated cytotoxicity against two human leukemic cell lines. Gong et al. simply do not teach that NK-92 cells are capable of lysing various tumor cells, including other leukemic tumor cells, of different origin or type. Klingemann Decl., ¶ 24a. As such, there is simply no teaching, suggestion, or motivation in Gong et al. that would lead one skilled in the art to use the NK-92 cell line *in vivo* to lyse tumor cells or as a cancer treatment, much less successfully reduce such a use to practice as a method of treating mammals. Id., ¶ 24c. Simply because a cell line is developed or established in the laboratory does not mean that there is an expectation of success for utilizing that cell line in a clinical setting. Certainly that was not the expectation with the NK-92 cell line because even the inventor did not initially recognize the importance or utility of the NK-92 cells in a clinical setting. Id.

2. Disclosure of Santoli et al.

Santoli et al. teach genetically modified cytotoxic T lymphoblastic leukemia (T-ALL) 104 and 107 cell lines and uses of these cell lines to treat cancer, both *in vivo* and *ex vivo*. Santoli et al., Abstract, 10:30-60. NK-92 cells are not disclosed by Santoli et al., nor is the use of NK-92 cells described. In fact, Santoli et al. do not provide a teaching, suggestion, or guidance with respect to any cell line other than T-ALL cells. In particular, Santoli et al. do not consider, teach, suggest, or provide guidance to NK-92 cells.

The Examiner misconstrues the teaching of Santoli et al. when, citing to Column 10 of Santoli et al., he states that "Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease or in preclinical in vivo studies." Final Office Action, ¶ 8. Rather, contrary to the Examiner's position, Santoli et al.'s teaching is limited to the use of "this invention," (i.e., T-ALL cells), not to all lytic human derived cell lines. The Examiner's conclusion is simply overly broad.

3. Santoli et al. is not relevant to Applicant's claimed method or combinable with Gong et al. because Santoli et al. disclose T-ALL cells which are structurally and functionally distinct

As set forth in the table below, the NK-92 cell line taught in Gong et al. or claimed by Applicant in independent claim 20 is structurally and functionally distinct from Santoli et al.'s T-ALL cell lines. Klingemann Decl., ¶ 28. As emphasized in Dr. Klingemann's declaration, know-how with respect to one cell line cannot automatically be transferred or applied to another cell line, even when the cells actually are closely related (which is not the case with NK-92 cells and T-ALL cells), including with respect to culture conditions, requirements for growth factors such as IL-2, survival and signaling patterns following adoptive transfer, ability to migrate to

tumor sites, sensitivity to chemotherapeutic agents, response to staining with vital dyes, ability to maintain their cytotoxic activity following radiation, and susceptibility to gene transfer. Id., ¶ 27. Furthermore, the know-how required to use a specific cell line as a method of treatment cannot automatically be transferred or applied to another cell line and is dependent on the distinguishing characteristics of each cell line. Id. Simply because one cell line has a specific utility does not mean that other closely related cell lines will have the same utility. Id. Each must be proven independently and the specific conditions necessary for successful results, including treatment, determined. Id.

Comparison of NK-92 cells to T-ALL cells

NK-92 Cells	T-ALL Cells
Derived from patient with aggressive LGL lymphoma	Derived from patient with T lymphoblastic leukemia
Originate from natural killer cells	Originate from T-cells
Do not require antibody stimulation in culture	Require antibody stimulation in culture
Maintain cytotoxicity and function after irradiation	Lose some cytotoxicity after irradiation
Have higher cytotoxicity than T-ALL cells	Have lower cytotoxicity than NK-92 cells

a. NK-92 cells and T-ALL cells were derived from different disease categories

The T-ALL cell line was derived from a patient with T lymphoblastic leukemia (T-ALL) (Santoli et al., 2:41-43), whereas the NK-92 cell line was derived from a patient with an aggressive LGL lymphoma. Klingemann Decl., ¶ 21. Leukemia and lymphoma are in different

disease categories and the cells derived therefrom are different cell lineages. Klingemann Decl., ¶ 28a. As such, the cell lines each have unique characteristics in culture and in undergoing proliferation. Id. As the inventor of the NK-92 cell line noted in his declaration, one skilled in the art would therefore assume that these two cell lines are different and that conclusions with respect to one of the cell lines cannot be drawn to the other cell line. Id.

b. NK-92 cells and T-ALL cells have different origins

T-ALL cells are of T-cell origin, are CD3-positive (a specific T-cell marker), CD8-positive, rearrange and express the T-cell receptor, are TCRαβ-positive, and are characterized by specific chromosomal translocations. *See* Santoli et al., 1:68, 2:14, and 4:27; *see also* Klingemann Decl., ¶ 28b. In addition, T-ALL cells lack natural cytotoxicity receptors such as NK-44 receptors that are found on NK-92 cells. In contrast, the NK-92 cell line is derived specifically from natural killer cells, making it a true NK cell line. Klingemann Decl., ¶ 28b. NK-92 cells are CD3-negative, CD8-negative, do not express or rearrange the T-cell receptor complex (TCR), and have different chromosomal rearrangements than T-ALL cells. Gong et al., 657-658; Klingemann Decl., ¶ 28b. As such, one cannot infer the behaviors, transfectability, or cytotoxic mechanisms of NK-92 cells from those of T-ALL cells because the cells have different phenotypes. Klingemann Decl., ¶ 28b.

c. NK-92 cells and T-ALL cells have different culture requirements

The culture for NK-92 cells is different from the culture for T-ALL cells. *See* Klingemann Decl., ¶ 28c. While T-ALL cells require antibody stimulation with CD2 or CD3 (a specific T cell marker) antigens to express IFN-γ, TNF-α, and GM-CSF (Santoli et al., 2:18, 47),

NK-92 cells do not require such antibody stimulation, but rather release these cytokines in response to stimulation by IL-2. Gong et al., 654; *see also* Klingemann Decl., ¶ 28d. Specifically, when NK-92 cells are cultured in α-minimum essential medium (α-MEM), the American Type Culture Collection (ATCC; Manassas, VA) recommends the media be supplemented with, among other things, 0.2 mM inositol, 0.1 mM 2-mercaptoethanol, 0.02 mM folic acid, 100-200 U/ml recombinant IL-2 (otherwise the cells die after 72 hours), and most surprisingly, a large proportion (25%) of two sera: 12.5% horse serum and 12.5% fetal bovine serum (FBS). In earlier passages, hydrocortisone is necessary. The cell density in culture is critical, and must be regularly checked and regulated by medium changes. The medium formulation, IL-2 concentration, serum concentration and cell density must be carefully regulated throughout the culture period. The culture of these cells are in stark contrast to other well-established cell lines (or even hybridomas), such as Madin-Darby Canine Kidney (MDCK) cells, which can thrive in simple MEM with 5% (FBS) and 2mM L-glutamine, 10mM N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), and sub-culturing once or twice a week.[2]

d. <u>NK-92 cells are more stable than T-ALL cells</u>

Additionally, NK-92 cells are more stable than T-ALL 104 cells. Tam et al. (Hum. Gene Ther., 10:1359-1373, 1999) have shown that NK-92 (both wild-type and transfected cells) cells require > 500 Gy to suppress proliferation, while Santoli et al., Cancer Res., 56:3021-3029, July 1996, reported that T-ALL 104 cells require 40 Gy irradiation to suppress proliferation.

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[2] Culture recommendations from the American Type Culture Collection (ATCC) for NK-92 cells are attached hereto.

Additionally, NK-92 cells maintain cytotoxicity and function even after irradiation, while T-ALL cells lose some cytotoxicity when irradiated. Klingemann Decl., ¶ 28e.

It has been reported that the standard treatment protocol for clinical trial in dogs required that the dogs be immunosuppressed using CsA, an immunosuppressive drug, starting the day before T-ALL 104 injections began and continuing through the first two weeks of T-ALL 104 injections. Santoli et al., Cancer Res., 56:3021-3029, July 1996. In contrast, NK-92 cells do not require supplemental immunosuppression. Klingemann Decl., ¶ 28f.

e. NK-92 cells have higher cytotoxic activity than T-ALL cells

Notably, comparative studies of NK-92 cells and T-ALL 104 cells further demonstrate that these cell lines are functionally quite different, with NK-92 cells having significantly higher cytotoxic activity than T-ALL 104 cells. For example, many hematological cancers are susceptible to killing by NK-92 cells, whereas these cancers are mostly resistant to lysis by T-ALL 104 cells. Klingemann Decl., ¶ 31. In fact, data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than T-ALL 104 cells or YT cells. See '955 Application, 35:11-36:20; 39:3-22; 44:11-28; Tables 5 and 6, Fig. 9. Even the inventor of the NK-92 cell line has indicated that the results demonstrating the superiority of the NK-92 cell line were surprising. Klingemann Decl., ¶ 33.

f. Summary

For at least these reasons, NK-92 cells are structurally and functionally different from the T-ALL cells disclosed by Santoli et al. One skilled in the art would therefore assume that conclusions with respect to one of these cell lines cannot be drawn to the other cell line. Klingemann Decl., ¶ 28a.

4. Applicant's method of treating a pathology as set forth in claim 20 is patentable over Gong et al. in view of Santoli et al. because Gong et al. merely established the NK-92 cell line and its phenotype while Santoli et al. is only relevant to T-ALL cells

Applicant disagrees with the Examiner's rejection of independent claim 20 for obviousness over Gong et al. in view of Santoli et al. because the combination of references fails to teach or suggest each and every element of Applicant's claimed method of treating a pathology *in vivo* by administering to the mammal NK-92 cells.

The Examiner has the burden pursuant to 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. In re Piasecki, 745 F.2d 1468 (Fed. Cir. 1984). To establish a *prima facie* case of obviousness, the Examiner must show: (i) a suggestion or motivation in the prior art, either from the references themselves or from generally available knowledge, for a person skilled in the art to choose the prior art reference or to combine the teachings of the references; (ii) a reasonable expectation of success; and (iii) that the reference or combination of references teach or suggest all of the claim limitations. See M.P.E.P. §§ 2141-2142; see also KSR Int'l Co. v. Teleflex, Inc., 127 S. Ct. 1727, 1741 (2007) (refusing to reject the use of teaching, suggestion, or motivation as a factor in the obviousness analysis because most inventions rely upon building blocks "long since uncovered, and claimed discoveries almost of necessity will be combinations of what, in some sense, is already known"). One court has noted that "[t]he KSR opinion only focused on the Federal Circuit's strict use of the [Teaching, Suggestion, Motivation] test in performing the obviousness analysis; it did not mention or affect the requirement that each and every claim limitation be found present in the combination of the prior art references before the analysis proceeds." Abbott Labs. V. Sandoz, Inc. 2007 U.S. Dist. Lexis 38216, *11 (N.D. Ill.

2007). Thus, KSR does not affect the Federal Circuit's holding that it is improper for the Examiner to use the applicant's invention as a blueprint to hunt through the prior art for the claimed elements and then combine them as claimed. See, e.g., In re Zurko, 111 F.3d 887 (Fed. Cir. 1997).

The Examiner has failed to meet his burden. Leaving aside the fact that Gong et al. limit their disclosure to establishing the NK-92 cell line, the differences between the NK-92 cells and T-ALL cells known at the time of filing Applicant's claimed method were so great that it was very unlikely that one skilled in the art would have found T-ALL cells to be any teaching with respect to NK-92 cells. The necessary nexus between the NK-92 cells taught by Gong et al. and an *in vivo* treatment of a pathology that would have led one skilled in the art to look to the teachings of Santoli et al. is missing. Simply because a cell line is developed or established in the laboratory does not mean that there is an expectation of success for utilizing that cell line in a clinical setting. Even the inventor of the NK-92 cell line did not initially recognize the importance or utility of the NK-92 cells in a clinical setting. Klingemann Decl., ¶ 24c. Additionally, the mere disclosure of NK-92 cells by Gong et al. is simply insufficient to obviate Applicant's claimed method and the Examiner's attempt to overcome the deficiencies of Gong et al. with the teachings of Santoli et al. is unfounded for a number of reasons, as detailed below.

First, any teaching, suggestion, or incentive in the prior art must not only motivate the skilled artisan to combine the teachings or suggestions, but must do so with a reasonable expectation of success. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art. In re Vaeck, 947 F.2d 488; M.P.E.P. § 2143.03. There is simply no such teaching, suggestion, or motivation in Gong et al.

to look to Santoli et al., let alone a reasonable expectation of success in combining those teachings. As set forth in detail above, the NK-92 cells disclosed by Gong et al. are phenotypically and functionally different from the T-ALL cells disclosed by Santoli et al. Because of these significant phenotypic and functional differences, there was simply no reason apparent to one skilled in the art at the time that Applicant's claimed method was filed to look to Santoli et al.'s teaching of T-ALL cells for any teaching with respect to a method of treating a pathology *in vivo* in a mammal by administering NK-92 cells, as is claimed by Applicant. Klingemann Decl., ¶ 29. Because of the significant and distinctive differences between these cell lines, the applicability and necessary requirements to use one of these cell lines as a method of treating *in vivo* is not applicable to the other, or to any other cell line for that matter. Id. Instead, the usefulness and necessary requirements for each would have to be characterized independently. Id. If one skilled in the art would have combined the teachings of Gong et al. and Santoli et al., the skilled artisan most certainly would not have had a reasonable expectation of success. Id., ¶ 30. In fact, the inventor of the NK-92 cell line has noted that application of the teachings of Santoli et al. to the NK-92 cells disclosed in Gong et al. would not have led to successful results because of the unique characteristics and requirements of the NK-92 cells. Id. Even with impermissible hindsight, one could not combine the teachings of Gong et al. and Santoli et al. to end up with Applicant's claimed method of treating a pathology *in vivo* by administering NK-92 cells because Applicant's NK-92 cell line is phenotypically and functionally different from Santoli et al.'s T-ALL cells.

Second, successful results and evidence of discovery further establish the patentability of Applicant's claimed method of treating a pathology *in vivo*. "[O]bjective evidence such as

commercial success, failure of others, long-felt need, and unexpected results must be considered before a conclusion on obviousness is reached." Minnesota Mining & Manufacturing Co. v. Johnson & Johnson Orthopedics, Inc., 976 F.2d 1559, 1573 (Fed. Cir. 1992) (noting the importance of secondary considerations in the obviousness analysis), citing Hybritech Inc. v. Monoclonal Antibodies, Inc., 802, F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986). Recent clinical trial studies demonstrated the "feasibility of large-scale expansion and safety of administering NK-92 cells as allogeneic cellular immunotherapy in advanced cancer patients and serves as a platform for future study of this novel natural killer (NK)-cell based therapy." Cytotherapy 10(6): 625-632, 2008. The methods used were tailored to NK-92 cells, which are very different from the methods tailored to T-ALL cells. Klingemann Decl., ¶ 35.

The Examiner alleges that:

> Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease whilst Gong et al. disclose that NK-92 cells are a lytic human derived cell line. In addition, as per the specification, page 2, last paragraph, use of NK cells and LAK cells to treat cancer in vivo was already known in the art. Gong et al. disclose that the NK-92 cell line displays characteristics of NK cells (see abstract), wherein use of NK cells to treat cancer in vivo was already known in the art.

Final Office Action, ¶ 8. The Examiner's conclusions are overly broad and misrepresent the disclosures of Santoli et al., Gong et al., and Applicant. Santoli et al. do not teach that all lytic human derived cell lines can be used *in vivo* to treat disease. Rather, Santoli et al. teach that T-ALL cells can be used in cancer therapy. See Santoli et al., 1:11-13. One skilled in the art would not extend such a limited teaching with respect to one cell line to be a teaching with respect to any other cell line. Klingemann Decl., ¶ 29. As discussed in detail above, there are significant phenotypic and functional differences between NK-92 cells and T-ALL cells, thereby

eliminating any reason for one skilled in the art at the time the claimed method was developed to look to Santoli et al.'s teaching of T-ALL cells to arrive at a method of treating a pathology *in vivo* in a mammal by administering NK-92 cells. Id., ¶ 29.

While the Examiner relies on Applicant's disclosure in the Specification (2:24-26) that "NK cells and LAK [lymphokine activated killer] cells have been used in both *ex vivo* therapy and *in vivo* treatment in patients with advanced cancer" to support his obviousness rejection, the Examiner fails to consider that NK cells and LAK cells are quite different from the claimed NK-92 cells and that Applicant's disclosure actually details the limitations of using NK and LAK cells *ex vivo* and *in vivo*. See '955 Application, 4:4-23. Applicant recognizes that "[t]here thus remains a need for a method of treating a pathology related to cancer or a viral infection with a natural killer cell line that maintains viability and therapeutic effectiveness against a variety of tumor classes." See '955 Application, 4:24-26. The Examiner has failed to recognize or consider that Applicant's claimed method, as set forth in claim 20, meets this need. See '955 Application, 5:4-5. While it was known in the art to use NK and LAK cells to treat a pathology, it was not known to use NK-92 cells for such a purpose until Applicant's claimed method was discovered. Gong et al.'s recognition in the Abstract that the novel NK-92 cell line "displays characteristics of activated NK-cells and could be a valuable tool to study their biology" does not impact the patentability of Applicant's claimed method because, at that time, there was absolutely no recognition that the NK-92 cells could be used *in vivo* as a method of treating, nor was there a motivation to look to Santoli et al. for such a teaching. Klingemann Decl., ¶ 29.

The Examiner goes on to support his rejection pursuant to 35 U.S.C. § 103(a) on the grounds that "in the post KSR Int'l Co. v. Teleflex Inc. universe, motivation per se is not even

required in a rejection under 35 U.S.C. § 103." Final Office Action, ¶ 8. Quoting KSR Int'l Co. v. Teleflex Inc., 550 U.S. m. 2007 WL 1237837 at 13 (2007), the Examiner states "if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill." Notably, the Examiner has acknowledged that "the two types of cells differ in phenotype" but has still concluded that "both the cells described by Santoli et al. and NK-92 are lytic human derived cell lines that can lyse various tumor cells." Final Office Action, ¶ 8. This conclusion is inaccurate because Gong et al. do not teach that NK-92 cells are capable of lysing various tumor cells of different origin or type. Klingemann Decl. ¶ 24. Instead, Gong et al. teach that NK-92 cells demonstrated cytotoxicity against two human leukemic cell lines in studies developed to characterize the newly isolated cell line. Id. Further, given that one skilled in the art would appreciate the significant phenotypic and functional differences between NK-92 cells and T-ALL cells, there would not have been any reason apparent to one skilled in the art at the time the claimed method was developed to look to Santoli et al.'s teaching of T-ALL cells to arrive at a method of treating a pathology *in vivo* in a mammal by administering NK-92 cells. Id., ¶¶ 27, 29. What the Examiner fails to appreciate is that Santoli et al. only teach methods applicable to T-ALL cells and do not provide guidance as to any other cell lines, while Gong et al. identify and partially characterize NK-92 cells which, at the time, was a new cell line. As discussed above, the inventor of the NK-92 cell line has noted that these two cell lines are from different cell lineages derived from different disease categories, leukemia and lymphoma. Id., ¶ 28. The T-ALL cell lines were derived from a patient with ALL, whereas the NK-92 cell line was derived from a patient with an aggressive LGL lymphoma. Id.

The actual application of a method for treating a pathology *in vivo* in a mammal by administering NK-92 cells would not have been obvious to a person of ordinary skill in the art based on the methods and teachings disclosed in Santoli et al. Id., ¶¶ 29, 30. The phenotypic and functional differences between the cells inherently prevent the know-how from one to be automatically transferred to the other, especially with any expectation of success. Id. Thus, contrary to the Examiner's conclusion, because Gong et al. do not teach a method of treating a pathology *in vivo*, it could not be obvious to use Gong et al. to arrive at, let alone improve, another technique.

The Examiner also asserts that "there is no teaching in Gong et al. that NK-92 cells are unacceptable for in vivo use." Final Office Action, ¶ 8. That notation, however, is irrelevant. It is the teaching of the reference that is relevant to an obviousness analysis, not what the reference does not teach. See, e.g., M.P.E.P. § 2143.01, citing KSR Int'l v. Teleflex Inc., 127 S.Ct. 1727, 1740-1741 (2007) (stating that "rejections on obviousness cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness"). Gong et al. do not teach or suggest that the NK-92 cells disclosed therein could be used *in vivo* to lyse tumor cells. Klingemann Decl., ¶ 24. This together with the fact that Santoli et al.'s teaching is limited to T-ALL cells renders the Examiner's combination of Gong et al. and Santoli et al. unsubstantiated.

The Examiner cites to M.P.E.P. § 2121, stating that "[w]hen the reference relied on expressly anticipates or makes obvious all of the elements of the claimed invention, the reference is presumed to be operable. Once such a reference is found, the burden is on the applicant to provide facts rebutting the presumption of operability." Final Office Action, ¶ 8. For the reasons set forth above, the Examiner has not established a *prima facie* case of obviousness.

Accordingly, the burden has not moved to Applicant to rebut the presumption of operability. However, even if the burden has moved to Applicant, the combination of Gong et al. and Santoli et al. would not have led to successful results because of the unique characteristics and requirements of these cells. Klingemann Decl., ¶ 30.

The Examiner also states that

> obviousness requires only a reasonable expectation of success. Regarding the Klingemann declaration, Santoli et al. teach that there is a need for cytotoxic cell lines which could be used to treat cancer. In view of the high level of skill in the art (Ph.D. or MD, with extensive research training) it would have been obvious to a routineer that other cytotoxic cell lines could be potentially used as per Santoli et al. In addition, the use of NK cells to treat cancer in vivo was already known in the art whilst Gong et al. disclose that the NK-92 cell line displays characteristics of NK cells.

Final Office Action, ¶ 8. As discussed above, there was not a reasonable expectation of success. As emphasized in the declaration of the inventor of the NK-92 cell line, the significant phenotypic and functional differences between NK-92 cells and T-ALL cells rendered the use of one of these cell lines as a method of treating *in vivo* inapplicable to the other, or to any other cell line for that matter, thereby precluding any expectation of success. Klingemann Decl., ¶¶ 29, 30. Additional comparative studies of NK-92 cells and TALL-104 cells further demonstrate that these cell lines are functionally quite different, with NK-92 cells having significantly higher cytotoxic activity than TALL-104 cells. Id., ¶ 31. For example, many hematological cancers are susceptible to killing by NK-92 cells, whereas these cancers are mostly resistant to lysis by TALL-104 cells. Id. In fact, data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than TALL-104 cells or YT cells. Id., ¶ 32. As further evidence of non-obviousness, the methods developed and being used in the clinic are very different for the two

cell lines. Reliance on the teachings of Santoli et al. would not have led to successful use of the NK-92 cells in a clinical setting. See, e.g., Id., ¶ 35.

With respect to Applicant's arguments that NK-92 cells and T-ALL cells are distinct cell lines, the Examiner alleges that Tam et al. state that "[a]n alternative is to use established cytotoxic NK tumor cell lines, which would give access to large numbers of effector cells. This concept has been proved by Cesano et al. (1997), who showed that an NK-like cell, TALL-104 was effective in treating a variety of malignancies in dogs." Final Office Action, ¶ 8. The Examiner continues: "contrary to the comments in the Klingemann declaration, Tam et al. disclose that TALL-104 is an NK-like cell line which is similar enough to NK cells that findings using TALL-104 cells can be extrapolated to NK cell lines." Final Office Action, ¶ 8. In fact, as set forth in Dr. Klingemann's declaration, Tam et al. actually demonstrate that NK-92 cells are more stable than T-ALL 104 cells. Klingemann Decl., ¶ 28e. Specifically, Tam et al. demonstrate that NK-92 cells and T-ALL cells are phenotypically distinct because Tam et al. showed that NK-92 cells require >500 Gy to suppress proliferation, while others have reported that T-ALL 104 cells require 40 Gy irradiation to suppress proliferation. See Santoli et al., Cancer Res., 56: 3021-3029, July 1996. Additionally, NK-92 cells maintain cytotoxicity and function even after irradiation, while T-ALL cells lose some cytotoxicity when irradiated. Klingemann Decl., ¶ 28e. NK-92 cells do not require supplemental immunosuppression. Id., ¶ 28f. Accordingly, T-ALL cells are immunogenic while NK-92 cells are not. Id.

The Examiner also alleges that "Klingemann et al. (1996) also disclose that NK-92 and TALL-104 cells have similar lytic properties." Final Office Action, ¶ 8. In fact, that is a misrepresentation of Klingemann et al. That reference actually acknowledges that "[a]

comparative study of the cytotoxic activity of the TALL-104 and the NK-92 cells has suggested, however, that NK-92 cells display a higher level of cytotoxicity than TALL-104 cells against leukemic and lymphoma targets and also lyse a broader spectrum of leukemic target cells including primary leukemias derived from patients." Klingemann et al., Biol. Blood Marrow Transplant., 2:68-75, 73 (1996). As set forth in detail above, data actually have demonstrated that NK-92 cells are, in fact, superior to T-ALL cells. See Klingemann Decl., ¶¶ 31-33.

The Examiner alleges that "there is no evidence of record that in vivo treatment with NK-92 cells is superior to in vivo treatment with TALL-104 cells." Final Office Action, ¶ 8. The Examiner is incorrect. See, e.g., Klingemann Decl., ¶¶ 31-33 (stating that "data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than TALL-104 cells or YT cells"). In fact, data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than T-ALL 104 cells or YT cells. See '955 Application, 35:11-36:20; 39:3-22; 44:11-28; Tables 5 and 6, Fig. 9.

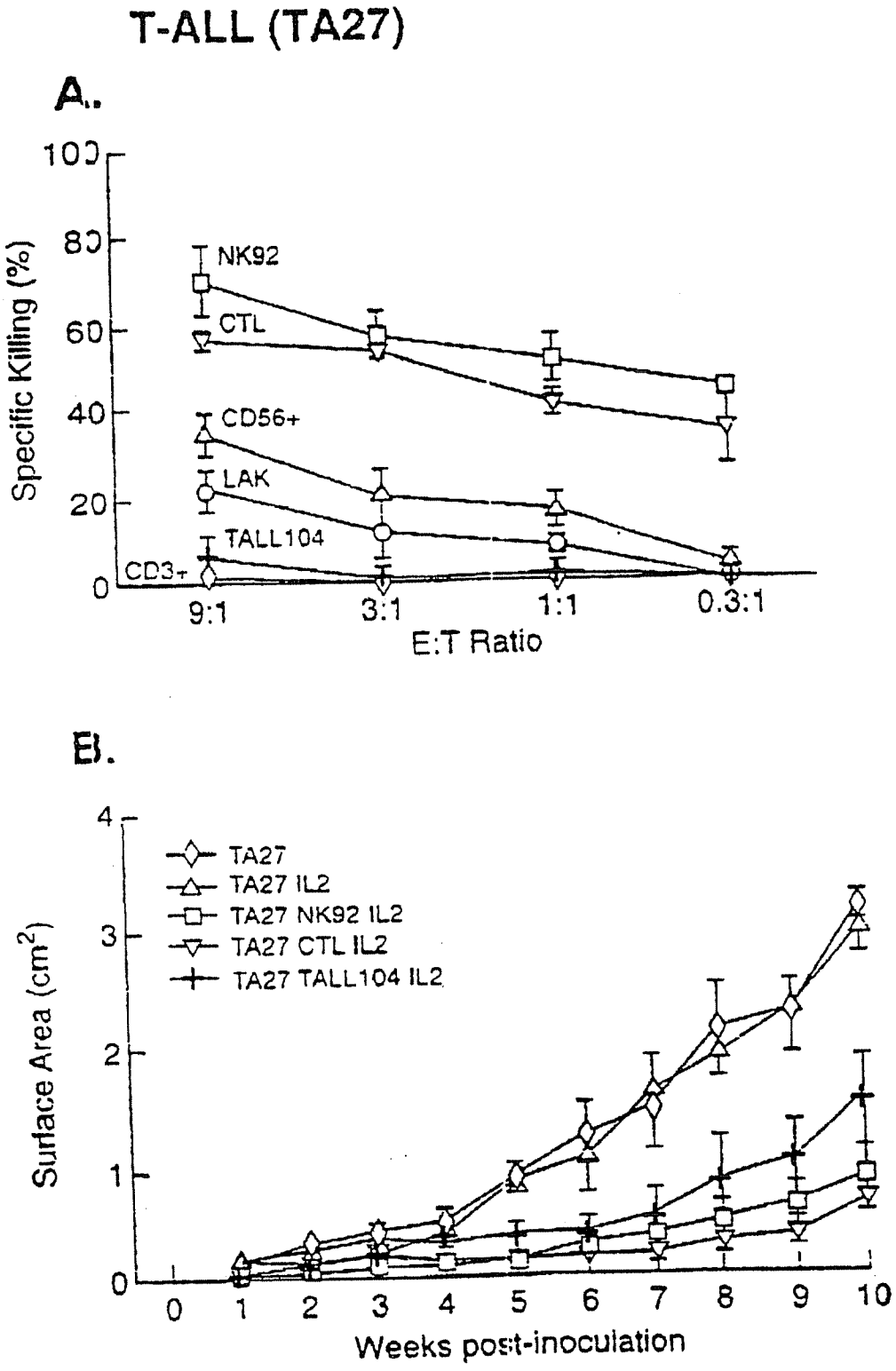
T-ALL (TA27)
A.
Specific Killing (%)
100
80
60
40
20
0
NK92
CTL
CD56+
LAK
TALL104
CD3+
9:1
3:1
1:1
0.3:1
E:T Ratio
B.
Surface Area (cm2)
4
3
2
1
0
TA27
TA27 IL2
TA27 NK92 IL2
TA27 CTL IL2
TA27 TALL104 IL2
0 1 2 3 4 5 6 7 8 9 10
Weeks post-inoculation

FIGURE 9

'955 Application, Fig. 9. These results demonstrate that the NK-92 cell line and the T-ALL 104 cell line are not even comparable. Klingemann Decl., ¶ 32. In fact, the inventor of the NK-92 cell line found these results to be surprising. Id., ¶ 33.

In fact, results recently published by the inventor are promising and encourage continued development of the use of NK-92 cells as a method of treatment. Klingemann Decl., ¶ 35. This study confirmed the feasibility of large-scale expansion and safety of administering *ex vivo* expanded NK-92 cells as allogeneic cellular immunotherapy in patients with refractory renal cell cancer and melanoma. See Arai et al., Cytotherapy, 10(6): 625-632 (2008) (a copy of which is attached hereto).

For at least the reasons discussed above, Gong et al. does not teach or suggest each and every element of Applicant's claimed method of treating a pathology. Because Gong et al. fail to teach or suggest each and every one of Applicant's claimed elements, Santoli et al.'s alleged teaching with respect to *in vivo* treatment by T-ALL cells becomes moot. The addition of Santoli et al. to Gong et al. does not ameliorate the deficiencies of Gong et al. as an obviating reference. Therefore, the rejection of claim 20 cannot stand.

5. Applicant's methods of treating a pathology as set forth in dependent claims 22, 26, 27, and 30 are also patentable over Gong et al. in view of Santoli et al.

The Examiner alleges that dependent claim 22, which depends from claim 20, is also obviated by the combination of Gong et al. in view of Santoli et al. Applicant disagrees with the Examiner's rejection of dependent claim 22 because dependent claim 22 also requires that the pathology is a cancer. Claim 22 is allowable by virtue of its dependency on claim 20, which is allowable for at least the reasons set forth above. *See* M.P.E.P. § 2143.03 (stating that "[i]f an

independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious"); see also In re Fine, 837 F.2d 1071, 5 USPQ2d 1506 (Fed. Cir. 1988). Accordingly, any teaching with respect to the pathology being a cancer is rendered moot because Gong et al. in view of Santoli et al. fail to teach or suggest each and every element of Applicant's claimed method treating a pathology for at least the reasons set forth above. The Examiner's rejection of claim 22 cannot stand.

The Examiner also alleges that dependent claim 26, which depends from claim 20, is also obviated by the combination of Gong et al. in view of Santoli et al. Applicant disagrees with the Examiner's rejection of dependent claim 26 because dependent claim 26 also requires that the cells be administered to a human intravenously. Claim 26 is allowable by virtue of its dependency on claim 20, which is allowable for at least the reasons set forth above. *See* M.P.E.P. § 2143.03 (stating that "[i]f an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious"); see also In re Fine, 837 F.2d 1071, 5 USPQ2d 1506 (Fed. Cir. 1988). Accordingly, any teaching with respect to the route of administration of the cells to the mammal being intravenous and the mammal being human is rendered moot because Gong et al. in view of Santoli et al. fail to teach or suggest each and every element of Applicant's claimed method treating a pathology for at least the reasons set forth above. The Examiner's rejection of claim 26 cannot stand.

The Examiner also alleges that dependent claim 27, which depends from claim 20, is also obviated by the combination of Gong et al. in view of Santoli et al. Applicant disagrees with the Examiner's rejection of dependent claim 27 because dependent claim 27 also comprises the step of administering to the mammal a cytokine that promotes the growth of NK-92 cells. Claim 27

is allowable by virtue of its dependency on claim 20, which is allowable for at least the reasons set forth above. *See* M.P.E.P. § 2143.03 (stating that "[i]f an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious"); see also In re Fine, 837 F.2d 1071, 5 USPQ2d 1506 (Fed. Cir. 1988). Santoli et al. do not disclose a method of treating comprising the step of administering to the mammal a cytokine that promotes the growth of NK-92 cells. Rather, Santoli et al. disclose "incorporating into the cell line a selected lymphokine gene." Santoli et al., 7:29-34. Thus, Santoli et al.'s teaching cannot obviate Applicant's dependent claim 27 because the combination of Gong et al. in view of Santoli et al. fail to teach or suggest Applicant's claimed method. The Examiner's rejection of claim 27 cannot stand.

The Examiner also alleges that dependent claim 30, which depends directly from claim 22 (and indirectly from claim 20), is also obviated by the combination of Gong et al. in view of Santoli et al. Applicant disagrees with the Examiner's rejection of dependent claim 30 because dependent claim 30 also requires that the cancer be a solid tumor. Claim 30 is allowable by virtue of its dependency on claim 20, which is allowable for at least the reasons set forth above. *See* M.P.E.P. § 2143.03 (stating that "[i]f an independent claim is nonobvious under 35 U.S.C. § 103, then any claim depending therefrom is nonobvious"); see also In re Fine, 837 F.2d 1071, 5 USPQ2d 1506 (Fed. Cir. 1988). Accordingly, any teaching with respect to the cancer being a solid tumor is rendered moot because Gong et al. in view of Santoli et al. fail to teach or suggest each and every element of Applicant's claimed method treating a pathology for at least the reasons set forth above. The Examiner's rejection of claim 30 cannot stand.

D. Conclusion

For at least the reasons set forth herein, Applicant respectfully requests that the Board reverse the Examiner's final rejection and allow all claims because the Examiner has failed to show or establish how Gong et al. in combination with Santoli et al. obviates Applicant's claimed invention. In accordance with the above remarks, claims 20, 22, 26, 27, and 30 are patentable over the cited references and allowance of same is hereby respectfully requested.

Applicant does not believe that a fee is due. However, if the Commissioner determines that a fee is required, the Commissioner is authorized to charge any required fee to Deposit Account No. 03-2026.

Respectfully submitted,

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VIII. CLAIMS APPENDIX

The following claims are the claims on appeal as presently amended:

1. (Withdrawn) A method of purging cells related to a pathology from a biological sample, said method comprising (i) obtaining a biological sample from a mammal, wherein the biological sample is suspected of containing cells related to the pathology, and (ii) contacting the biological sample with a medium comprising NK-92 or modified NK-92 natural killer cells, wherein the modified NK-92 cells have been modified by a physical treatment or by transfection with a vector; whereby the natural killer cells purge cells related to the pathology from the sample.

2. (Withdrawn) The method described in claim 1 wherein the pathology is a cancer.

3. (Withdrawn) The method described in claim 1 wherein the pathology is an infection by a pathogenic virus.

4. (Withdrawn) The method described in claim 3 wherein the pathogenic virus is human immunodeficiency virus, Epstein-Barr virus, crytomegalovirus, or herpes virus.

5. (Withdrawn) The method described in claim 1 wherein the biological sample is human blood or bone marrow.

6. (Withdrawn) The method described in claim 1 wherein the natural killer cell is immobilized on a support.

7. (Withdrawn) The method described in claim 1 wherein the modified NK-92 cells have been modified by a physical treatment that renders them non-proliferative, said treatment not significantly diminishing their cytotoxicity, by treatment that inhibits express of HLA antigens on the NK-92 cell surface, by transfectoin with a vector, or by any combination thereof.

8. (Withdrawn) The method described in claim 7 wherein the cells have been transfected with a vector encoding a cytokine that promotes the growth of the cells, a vector encoding a protein that is responsive to an agent, a vector encoding a cance cell receptor molecule, or with any combination thereof.

9. (Withdrawn) The method described in claim 1 wherein the medium further comprises cytokine that promotes the growth of the cells.

10. (Withdrawn) A method of treating a pathology ex vivo in a mammal comprising the steps of:

(i) obtaining a biological sample from the mammal, wherein the sample is suspected of containing cells related to the pathology;

(ii) contacting the biological sample with a medium comprising NK-92 or modified NK-92 natural killer cells, wherein the modified NK-92 cells have been modified by a physical treatment or by transfection with a vector, whereby the cells related to the pathology in the sample are selectively destroyed, thereby producing a purged sample; and

(iii) returning the purged sample to the mammal.

11. (Withdrawn) The method described in clam 10 wherein the pathology is a cancer.

12. (Withdrawn) The method described in claim 11 wherein the cancer is a leukemia, a lymphoma or a multiple myeloma.

13. (Withdrawn) The method described in claim 10 wherein the pathology is an infection by a pathogenic virus.

14. (Withdrawn) The method described in claim 13 wherein the pathogenic virus is human immunodeficiency virus, Epstein-Barr virus, cytomegalovirus, or herpes virus.

15. (Withdrawn) The method described in claim 10 wherein the biological sample is blood or bone marrow and wherein the mammal is a human.

16. (Withdrawn) The method described in claim 10 wherein the natural killer cell is immobilized on a support.

17. (Withdrawn) The method described in claim 10 wherein the medium comprises modified NK-92 cells which have been modified by a physical treatment that renders them non-proliferative, said treatment not significantly diminishing their cytotoxicity, by treatment that inhibits expression of HLA antigens on the NK-92 cell surface, by transfection with a vector, or by any combination thereof.

18. (Withdrawn) The method described in claim 17 wherein the cells have been transfected with a vector encoding a cytokine that promotes the growth of the cells, a vector encoding a protein that is responsive to an agent, a vector encoding a cancer cell receptor molecule, or with any combination thereof.

19. (Withdrawn) The method of treating a cancer described in claim 10 wherein the medium further comprises a cytokine that promotes the growth of the cells.

20. (Previously presented) A method of treating a pathology *in vivo* in a mammal comprising the step of administering to the mammal a medium comprising NK-92 cells (available from American Type Culture Collection (ATCC) as Deposit No. CRL-2407).

21. (Withdrawn) The method described in claim 20 wherein the modified NK-92 cells have been transfected with a vector encoding a cytokine that promotes the growth of the cells, with a vector encodinga protein that is responsive to an agent, a vector encoding a cancer cell receptor molecule, or with any combination thereof.

22. (Previously presented) The method described in claim 20 wherein the pathology is a cancer.

23. (Withdrawn) The method of treating a pathology described in claim 31 wherein the cancer is a leukemia, a lymphoma or a multiple myeloma.

24. (Withdrawn) The method described in claim 20 wherein the pathology is an infecton by a pathogenic virus.

25. (Withdrawn) The method described in claim 24 wherein the pathogenic virus is human immunodeficiency virus, Epstein-Barr virus, cytomegalovirus, or herpes virus.

26. (Previously presented) The method of treating a pathology described in claim 20 wherein the route of administration of the cells to the mammal is intravenous and the mammal is human.

27. (Previously presented) The method of treating a pathology described in claim 20 further comprising the step of administering to said mammal a cytokine that promotes the growth of said NK-92 cells.

28. (Withdrawn) The method of treating a pathology decribed in claim 26 wherein the NK-92 is modified by transfection with a vector encoding a protein that is responsive to an agent such that when the agent is taken up by the cell, the cell is inactivated, and wherein the method further comprises administering to the mammal, after a time sufficient for the natural killer cell to treat the cancer has elapsed, an amount of the agent effective to inactivate the cell.

29. (Withdrawn) The method of treating a pathology described in claim 28 wherein the agent is acyclovir or gancyclovir.

30. (Previously presented) The method of treating a pathology described in claim 22 wherein the cancer is a solid tumor.

31. (Withdrawn) The method of treating a pathology described in claim 22 wherein the cancer is a non-solid tumor of circulating cells.

IX. EVIDENCE APPENDIX

(1) Declaration of Hans Klingemann, M.D., Ph.D. Pursuant to 37 C.F.R. § 1.132, filed on October 15, 2008, in support of the Request for Continued Examination filed on October 15, 2008, in response to the Final Office Action mailed on April 15, 2008.

(2) Arai et al., Cytotherapy, 10(6): 625-632 (2008).

(3) Culture recommendations from the American Type Culture Collection (ATCC) for NK-92 cells.

Appendix A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Hans Klingemann	
Serial No. 10/008,955	**NATURAL KILLER CELL LINES AND METHODS OF USE**
Filed: December 7, 2001	
Art Unit: 1644	
Patent Examiner: Ronald B. Schwadron	
Attorney Docket No. 06-129PCT/US/CIP	
Confirmation No.: 5420	

DECLARATION OF HANS KLINGEMANN, M.D., Ph.D.
PURSUANT TO 37 C.F.R. § 1.132

I, Hans Klingemann, M.D., Ph.D., of Boston, Massachusetts, hereby declare that:

1. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

2. I am the sole inventor of the modified NK-92 cells disclosed in U.S. Patent Application Serial No. 10/008,955 (hereinafter, "the '955 Application"), identified above.

3. I submit this Declaration in support of the Response To Final Office Action filed on October 15, 2008.

4. I earned my Vor-Diplom in Biology from the University of Heidelberg, Heidelberg, Germany, in 1971, and my M.D. from the University of Wurzburg Medical School, Germany, in 1976. I carried out my internship in Internal Medicine and Surgery at the University of Wurzburg Medical School, Germany, from 1977-1978 and my residency in Internal Medicine at the University of Marburg Medical School, Germany, from 1978-1984. I received additional Post-graduate training in Bone Marrow Transplant/Oncology at the Fred Hutchinson Cancer Research Center, Seattle, WA, from 1984-1986.

5. I have held academic appointments at the University of Marburg Medical School (Privat-Dozent of Medicine, 1983-1986; Professor of Medicine, 1986-1987), University of British Columbia, Vancouver, CDN (Clinical Associate Professor, 1987-1995; Clinical Professor, 1995-1997), RUSH Medical College, Chicago, IL (Coleman Foundation Professor of Medicine, 1997-2004), and TUFTS University School of Medicine, Boston, MA (Professor of Medicine, 2004-present).

6. I have also held hospital/research appointments at the following facilities: Fred Hutchinson Cancer Research Center, Seattle, WA (Research Associate, 1984-1986); University of Marburg Medical School, Germany (Attending Physician, Dept. of Medicine, 1986-1987); Vancouver Hospital and Health Sciences Center, Vancouver CDN (Active Staff, Div. Of Hematology, 1987-1997); British Columbia Cancer Agency, Vancouver CDN (Active Staff, Clinical Hematology, 1987-1997); Vancouver Hospital

and BC Cancer Center, CDN (Attending Physician, Div. Of Hematology, 1987-1997); Leukemia/Bone Marrow Transplant Program of BC (Member, 1987-1997); Terry Fox Laboratory for Hematology/Oncology, BC Cancer Research Center, Vancouver, CDN (Chief, Transplantation Biology Laboratory, 1990-1997); RUSH University Medical Center, Chicago, IL (Director, Section of Bone Marrow Transplant & Cell Therapy, 1997-2004; Medical Director, Sramek Center for Cell Engineering, 2001-2004); TUFTS-New England Medical Center, Boston, MA (Senior Investigator, Molecular Oncology Research Institute, 2005-present; Director, Bone Marrow and Hematopoietic Cell Transplant Program, 2004-present); and TUFTS-NEMC Cancer Center, Boston, MA (Director, Hematologic Malignancy Program, 2007-present).

7. Additionally, I have advised numerous trainees over the course of my academic and professional careers and have taught numerous classes, both at the undergraduate and graduate levels.

8. Over the course of my career, my research projects have included studying various basic and clinical issues in transplantation immunology covering areas such as dendritic vaccines, natural killer cell biology and mesenchymal stem cells. This translational research has resulted in over 150 publications and a variety of innovative clinical trials.

9. I have authored numerous peer-reviewed publications, review papers/editorials, non-peer reviewed publications/conference proceedings, books and book chapters, and abstracts in the fields of translational research, transplantation biology, and tumor

immunology, including a number of publications relating to natural killer cells and NK-92 cells. A list of my publications is attached hereto as Exhibit 1.

10. I have also been invited to make numerous oral presentations to a variety of audiences on topics related to the fields of translational research, transplantation biology, and tumor immunology. A list of my oral presentations is included in Exhibit 1 hereto.

11. I am also a member of the following professional associations:

International Society of Experimental Hematology
American Society of Hematology
International Society for Cell Therapy
American Society for Blood and Bone Marrow Transplantation
American Society for Clinical Oncology.

12. Over the course of my academic and professional careers, I have received numerous awards and honors for my research contributions, including:

Dr. *Med.* (Magna Cum Laude)
Wolf Boas Research Award by the German Society of Gastroenterology for the best Doctoral Thesis
Habilitation (prerequisite for full professorship), University of Wurzburg Medical School, German (Ph.D. equivalent)
German Cancer Research Foundation Fellowship

13. My education, training, laboratory research, teaching experiences, and professional activities have enabled me to develop an expertise in various specialties within the field of translational research, transplantation biology, and tumor immunology, including an expertise on natural killer cells and NK-92 cells, and their use in the treatment of cancers and viruses.

14. Based on my educational background and work experience, I consider myself to be one skilled in the arts of translational research, transplantation biology, and tumor immunology, and particularly in the area of natural killer cells and NK-92 cells.

15. I am the inventor of the modified NK-92 cell line disclosed and claimed in the '955 Application.

16. I have read and am familiar with the '955 Application as it was filed in the U.S. Patent and Trademark Office and the claims of that application as currently pending in the Response To Final Office Action filed herewith.

17. I have reviewed the following prior art references cited by the Examiner of the '955 Application in the Final Office Action mailed on April 15, 2008, and am familiar with the material disclosed therein:

(a) Gong et al., Leukemia, 1994 (hereinafter, "Gong et al."); and

(b) U.S. Patent No. 5,272,082 to Santoli et al. (hereinafter, "Santoli et al.").

18. I am one of the authors of Gong et al. and am the sole inventor of the immortal cell line, NK-92, disclosed therein.

19. I have reviewed the Final Office Action issued for the '955 Application, which was mailed on April 15, 2008 (hereinafter, "Office Action"), and which contains the following statements:

> It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have created the claimed invention because Gong et al. teach use of NK-92 cells, while Santoli et al. teach in vivo use of cytotoxic cell lines. One of ordinary skill in the art would have been motivated to do so because Santoli et al. teach that lytic human derived cell lines can be used in vivo to treat disease or in preclinical in vivo studies (see column 10).

Office Action, ¶ 10.

20. The Examiner's statements are incorrect in view of the state of the tumor immunology art at the time that I invented the method of treating a pathology *in vivo* in a mammal by administering NK-92 cells, disclosed and claimed in the '955 Application. One skilled in the art would *not* have combined either Gong et al. with Santoli et al. at that time for at least the reasons set forth in paragraphs 21-40, *infra*.

21. Gong et al. disclosed the NK-92 cell line that I established from peripheral blood mononuclear cells of a fifty-year-old male patient who was diagnosed with an aggressive LGL lymphoma in 1992.

22. At the time that Gong et al. was written, I thought that the NK-92 cell line provided a suitable model to study the biology of NK-cells and activated NK-cells.

23. All experiments disclosed in Gong et al. were performed *in vitro*. Gong et al. partially characterized the cytotoxic profile of NK-92 cells.

24. The Examiner's characterization of Gong et al. is incorrect for at least the following reasons:

a. The Examiner incorrectly states that "Gong et al. teach use of NK-92 cells to lyse leukemic tumor cells." *See* Office Action, ¶ 10. Rather, Gong et al. teach that NK-92 cells demonstrated cytotoxicity against two human leukemic cell lines, but do not teach that NK-92 cells are capable of lysing various tumor cells, including other leukemic tumor cells, of different origin or type.

c. While Gong et al. do not specifically teach that NK-92 cells are unacceptable for *in vivo* use, there is no teaching, suggestion, or motivation in Gong et al. that would lead one skilled in the art to use the NK-92 cell line *in vivo* to lyse tumor cells or as a cancer treatment, much less successfully reduce such a use to practice as a method of treating mammals. In fact, I did not initially recognize the importance or utility of the NK-92 cell line in a clinical setting.

25. Santoli et al. disclose genetically modified cytotoxic T lymphoblastic leukemia cell lines (T-ALL) 104, 107 and 103/2 and their use to treat cancer, both *in vivo* and *ex vivo*. The disclosure in Santoli et al. is limited to T-ALL cells. There is absolutely no teaching or suggestion in Santoli et al. with respect to cell lines in general, or with respect to NK-92 cells in particular, nor is their use described.

26. In fact, I was not aware of Santoli et al.'s T-ALL cell lines at the time that I created the unmodified NK-92 cell line (available from American Type Tissue Collection (ATCC) as Deposit No. CRL-2407) disclosed in Gong et al. or at the time that I arrived at the method of treating a pathology *in vivo* in a mammal by administering NK-92 cells disclosed in the '955 Application.

27. As one skilled in the art, it has been my experience that know-how with respect to one cell line cannot automatically be transferred or applied to another cell line, even where the cells are closely related, including with respect to culture conditions, requirements for growth factors such as IL-2, survival and signaling patterns following adoptive transfer, ability to migrate to tumor sites, sensitivity to chemotherapeutic agents, response to staining with vital dyes, ability to maintain their cytotoxic activity following

radiation, and susceptibility to gene transfer. Furthermore, the know-how required to use a specific cell line as a method of treatment cannot automatically be transferred or applied to another cell line and is dependent on the distinguishing characteristics of each cell line. Simply because one cell line has a specific utility does not mean that other closely related cell lines will have the same utility. Each must be proven independently and the specific conditions necessary for successful results, including treatment, determined.

28. In fact, as set forth below, the T-ALL cell line is not even comparable or related to the NK-92 cell line that I developed and disclosed in Gong et al. Accordingly, there was no reason apparent to one skilled in the art at the time I arrived at the claimed method of treating a pathology *in vivo* in a mammal by administering NK-92 cells to look to Santoli et al.'s teaching of of T-ALL cells for any teaching with respect to methods of treatment with NK-92 cells.

a. The T-ALL cell lines were derived from a patient with ALL, whereas the NK-92 cell line was derived from a patient with an aggressive LGL lymphoma. These two diseases, leukemia and lymphoma, are in different disease categories and the cells derived therefrom are different cell lineages. As such, the cell lines each have unique characteristics in culture and in undergoing proliferation. One skilled in the art would therefore assume that these two cell lines are different and that conclusions with respect to one of the cell lines cannot be drawn to the other cell line.

b. T-ALL cells are of T-cell origin, are CD3-positive (a specific T-cell marker), CD8-positive, rearrange and express the T-cell receptor, are TCRαβ-positive, and are characterized by specific choromosomal translocations. *See* Santoli et al., 1:68, 2:14, and 4:27. In addition, T-ALL cells lack natural cytotoxicity receptors such as NK-44 receptors that are found on NK-92 cells. In contrast, the NK-92 cell line is a true NK cell line (i.e., it is derived specifically from natural killer cells). NK-92 cells are CD3-negative, CD8-negative, do not express or rearrange the T-cell receptor complex (TCR), and have different chromosomal rearrangements than T-ALL cells. As such, one cannot infer the behaviors, transfectability, or cytotoxic mechanisms of NK-92 cells from those of T-ALL cells because the cells have different phenotypes.

c. NK-92 cells have unusual requirements for sub-culturing. Specifically, when cultured *in vitro* in α-minimum essential medium (α-MEM), the American Type Culture Collection (ATCC; Manassas, VA) recommends the media be supplemented with, among other things, 0.2 mM inositol, 0.1 mM 2-mercaptoethanol, 0.02 mM folic acid, 100-200 U/ml recombinant IL-2 (otherwise the cells die after 72 hours), and most surprisingly, a large proportion (25%) of two sera: 12.5% horse serum and 12.5% fetal bovine serum (FBS). In earlier passages, hydrocortisone is necessary. The cell density in culture is critical, and must be regularly checked and regulated by medium changes. The medium formulation, IL-2 concentration, serum concentration and cell density must be carefully regulated throughout the culture period. The culture of these cells is in

contrast to T-ALL cells, which require fetal bovine serum for growth and proliferation, and is similar to other well-established cell lines (or even hybridomas), such as Madin-Darby Canine Kidney (MDCK) cells, which can thrive in simple MEM with 5% (FBS) and 2mM L-glutamine, 10mM N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), and sub-culturing once or twice a week.

d. Santoli et al. teach that T-ALL cells require antibody stimulation with CD2 or CD3 (a specific T cell marker) antigens to express (IFN)-γ, TNF-α, and GM-CSF. *See* Santoli et al. 2:18, 2:47. NK-92 cells do not require antibody stimulation to express (IFN)-γ, TNF-α, and GM-CSF, but rather release these cytokines in response to stimulation by IL-2.

e. Additionally, NK-92 cells are more stable than TALL-104 cells. Tam et al. (Hum. Gene Ther., 10: 1359-1373, 1999) have shown that NK-92 (both wild-type and transfected cells) cells require > 500 Gy to suppress proliferation, while Santoli et al. reported that TALL-104 cells require 40 Gy irradiation to suppress proliferation (*see* Santoli et al., Cancer Res., 56: 3021-3029, July 1996). Additionally, NK-92 cells maintain cytotoxicity and function even after irradiation, while T-ALL cells lose some cytotoxicity when irradiated.

f. Santoli et al. also reported that the standard treatment protocol for clinical trial in dogs required that the dogs be immunosuppressed using CsA, an immunosuppressive drug, starting the day before TALL-104 injections began and continuing through the first two weeks of TALL-104 injections. *See* Santoli et al.,

Cancer Res., 56: 3021-3029, July 1996). NK-92 cells do not require supplemental immunosuppression. These data suggest that TALL-104 cells are immunogenic while NK-92 cells are not.

29. Accordingly, given these significant phenotypic and functional differences between NK-92 cells and T-ALL cells, there was no reason apparent to one skilled in the art at the time I developed the method of treating a pathology *in vivo* in a mammal by administering NK-92 cells to look to Santoli et al.'s teaching of T-ALL cells to arrive at similar method of treatments. Because of the distinctive differences between these cell lines, the applicability and necessary requirements to use one of these cell lines as a method of treating *in vivo* is not applicable to the other, or any other cell line for that matter. The usefulness and necessary requirements for each would have to be characterized independently.

30. For at least the reasons set forth in paragraphs 21-29, *supra*, it would not have been obvious to one skilled in the art at the time the method of treating a pathology *in vivo* in a mammal by administering NK-92 cells was made to have combined the teachings of Gong et al. with Santoli et al. Most certainly one skilled in the art would not have had a reasonable expectation of success. If one skilled in the art were to have applied the teachings of Santoli et al to the NK-92 cells disclosed in Gong et al, they would not have had successful results because of the unique characteristics and requirements of these cells.

31. Additional comparative studies of NK-92 cells and TALL-104 cells further demonstrate that these cell lines are functionally quite different, with NK-92 cells having

significantly higher cytotoxic activity than TALL-104 cells. For example, many hematological cancers are susceptible to killing by NK-92 cells, whereas these cancers are mostly resistant to lysis by TALL-104 cells.

32. In fact, data disclosed in the '955 Application demonstrate that NK-92 cells are more cytolytic than TALL-104 or YT cells. *See* '955 Application, Tables 5 and 6, Fig. 9.

33. Notably, the results demonstrating that the NK-92 cell line is a superior cell line to the TALL-104 cell line were surprising.

34. Given the significant phenotypic and functional differences between NK-92 cells and T-ALL cells and the cytotoxic superiority of NK-92 cells to TALL-104 cells, there was no reason apparent to one skilled in the art as of the filing date of the '955 Application to look to Santoli et al.'s teaching of TALL cells for treatment of disease for any teaching with respect to the NK-92 cells disclosed in Gong et al.

35. Neither of the references cited by the Examiner in the Final Office Action, either alone or in combination, teach or suggest the method of treatment with NK-92 cells disclosed and claimed in the '955 Application and therefore these references do not obviate the claimed method of treating a pathology *in vivo* in a mammal by administering NK-92 cells. In fact, we recently published in Cytotherapy (10(6): 625-632, 2008) Phase I trial results using NK-92 cells based on methods tailored to NK-92 cells, which are very different from methods tailored to TALL cells, and not disclosed or suggested in Santoli et al or Gong et al. *See* Exhibit 2 attached hereto. The results are promising and encourage continued development of the use of NK-92 cells as a method of treatment.

COPY

36. Signed at Boston, MA, this 15 day of Oct., 2008.

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EXHIBIT 1

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EXHIBIT 2

NK-92 phase I trial

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Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial

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Background

Renal cell cancer and malignant melanoma are two types of cancer that are responsive to immunotherapy. In this phase I dose-escalation study, the feasibility of large-scale expansion and safety of administering ex vivo*-expanded NK-92 cells as allogeneic cellular immunotherapy in patients with refractory renal cell cancer and melanoma were determined.*

Methods

Twelve patients (aged 31–74 years) were enrolled, three per cohort at cell dose levels of $1 \times 10^8/m^2$, $3 \times 10^8/m^2$, $1 \times 10^9/m^2$ and $3 \times 10^9/m^2$. One treatment course consisted of three infusions. Eleven patients had refractory metastatic renal cell cancer; one patient had refractory metastatic melanoma.

Results

The NK-92 cells were expanded in X-Vivo 10 serum-free media supplemented with 500 U/mL Proleukin recombinant human interleukin-2 (rhIL-2), amino acids and 2.5% human AB plasma. Final yields of approximately 1×10^9 cells/culture bag (218–250 × expansion) over 15–17 days were achievable with ≥ 80% viability. Infusional toxicities of NK-92 were generally mild, with only one grade 3 fever and one grade 4 hypoglycemic episode. All toxicities were transient, resolved and did not require discontinuation of treatment. One patient was alive with disease at 4 years post-NK-92 infusion. The one metastatic melanoma patient had a minor response during the study period. One other patient exhibited a mixed response.

Discussion

This study establishes the feasibility of large-scale expansion and safety of administering NK-92 cells as allogeneic cellular immunotherapy in advanced cancer patients and serves as a platform for future study of this novel natural killer (NK)-cell based therapy.

Keywords

cancer, cell therapy, NK-92, phase I.

Introduction

Treatment options remain very limited for patients with metastatic renal cancer and metastatic melanoma. Median survival is 7–10 months for metastatic renal cancer and metastatic melanoma and both diseases are resistant to chemotherapy and/or radiotherapy [1]. Both cancers, however, seem to be responsive to immunotherapy [2–4] and cellular immunotherapy is increasingly being considered as a form of treatment that is non-cross-reactive with prior chemotherapy and radiation [5,6].

Natural killer (NK) cells are particularly attractive for adoptive cellular immunotherapy because of their unique ability to lyse target cells without priming [7]. Autologous NK cells from cancer patients, however, may be dysfunctional and may not recognize the malignant target. Autologous NK cells may also be inhibited by 'self' HLA expression and some tumors may in fact express functional HLA antigens (Ag) capable of inhibiting NK cell function. Allogeneic NK cells, therefore, potentially represent a better NK cell product for immunotherapy. NK-92 is a human NK-cytotoxic cell line that represents a pure allogeneic activated NK cell source. NK-92 is interleukin-2 (IL-2) dependent, lacks killer cell inhibitory receptors (KIR) and is broadly cytotoxic against a variety of hematologic and solid tumor cell lines, including leukemia, lymphoma, malignant melanoma, prostate cancer and

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breast cancer [8]. *Ex vivo* expansion of NK-92 under good tissue practice (GTP) conditions for clinical use has allowed its entry into phase I study as a novel immunotherapy in advanced cancers [9]. The NK-92 cell line is originally derived from a non-Hodgkin's lymphoma with large granular lymphocyte morphology and a $CD56^+CD3^-CD16^-$ immunophenotype. Studies in SCID mice have confirmed that NK-92 inoculation itself is not leukemogenic. The tumoricidal activity of NK-92 against human leukemias has been tested *in vitro* against leukemic cell lines and primary leukemia cells, as well as *in vivo* by adoptive transfer of NK-92 cells into xenografted SCID mice, with the result of prolonged survival and no signs of leukemia development [10]. NK-92 infusion has further been found to prolong survival in SCID mice inoculated with human malignant melanoma cells, an observation that served as the basis for this clinical trial [11].

The objective of this study was to determine the safety of infusing NK-92 cells in patients with advanced renal cell cancer and melanoma. The three infusions, each given 48 h apart, had no severe side-effects and several patients showed objective anti-tumor responses, suggesting further exploration of this cellular treatment modality in selected cancer indications is warranted.

Methods

Patient eligibility

The study was open from April 2002 to June 2004 at Rush University Medical Center (Chicago, IL, USA). The protocol was approved by the Institutional Review Board and had obtained FDA investigational new drug application status for the *ex vivo* expansion of NK-92 cells. All patients signed informed consent before any study-related procedures. Patients with histologically confirmed metastatic renal cell cancer or malignant melanoma refractory to, or having failed, standard therapy, including surgery, radiation and chemotherapy, were eligible for treatment on this protocol. All patients had measurable disease [by computed tomography (CT) scan or physical examination] and had undergone several prior treatments, including high-dose IL-2 therapy and allogeneic stem cell transplant (SCT). Other eligibility criteria included ECOG 0 or 1, white blood cells (WBC) $> 2.0 \times 10^9$/L, Hb > 8 g/dl, platelets $\geq 75 \times 10^9$/L, creatinine < 2.0 mg/dL and total bilirubin < 2.0 mg/dL. Exclusion criteria included ECOG ≥ 2 and concurrent treatment with corticosteroids and/or other immunosuppressive drugs.

Trial design

The trial was a single-center, open-label, dose-escalation study. Three patients were treated at each dose level: 1×10^8 cells/m^2, 3×10^8cells/m^2, 1×10^9 cells/m^2 and 3×10^9cells/m^2. One treatment course consisted of three infusions of the cell dose over 48 h. Infusion days were designated as days 1, 3 and 5. The rationale for the schedule was to infuse as many NK-92 cells before a T-cell directed immune response would theoretically occur.

Manufacturing of the NK-92 cell product

Manufacturing of clinical-grade NK-92 cells was performed under GTP conditions at the Sramek Center for Cell Engineering at Rush University Medical Center [9]. At 3 weeks before the targeted date of infusion, NK-92 cell cultures were initiated from the NK-92 Working Cell Bank. NK-92 cells were expanded in X-Vivo 10 serum-free medium supplemented with 500 U/mL Proleukin recombinant human (rh)IL-2, 0.6 mm l-asparagine, 3 mm l-glutamine, 1.8 mm l-serine and 2.5% human AB plasma. The cultures were initiated at 2.5×10^5 cells/mL in 25 mL (6.25×10^6 cells) in 1-L Vuelife culture bags (American Fluoroseal Corp., Gaithersburg, MD, USA), with the addition of media every 3 days, maintaining a density of 2.5×10^5 cells/mL, and with daily mild disruption of cell aggregates. Final yields of approximately 1×10^9 cells/culture bag (218–250-fold expansion) over 15–17 days was achievable, with $\geq 80\%$ viability. After quality control verification and quality assurance release that included Gram stain, culture and mycoplasma testing, the final NK-92 cell product was resuspended in GM-2 medium (Plasma-Lyte-A medium supplemented with 2.5% human AB plasma) and infused fresh. The last feeding with rhIL-2 and fresh medium was 48 h before the first day of infusion of the expanded NK-92 product. In addition, after completion of the cell culture period, a standard cytotoxicity assay was performed to assess the functional capacity of the *ex-vivo*-expanded NK-92 cells. Calcein AM-labeled K562 and Raji cells were used as targets to determine NK-92 cell cytotoxicity of the *ex vivo*-expanded cells. The NK-92 cells were irradiated with 1000 cGy prior to infusion into the patient (Cesium Source-Blood Bank, Rush University Medical Center).

On the day of infusion, hydration (200 mL NS/h) was given to the patient 2 h prior to the NK-92 cell infusion and continued for 2 h after NK-92 infusion. The total volume of the NK-92 cell product infusate was

100–200 mL, depending on the body weight of the individual patient. The cells were infused at a rate of 5 mL/min, with a total infusion time of approximately 20–30 min. All patients received premedication with diphenhydramine before the start of each cell infusion.

Of note, the NK-92 cell line was being commercialized during the course of the clinical trial.

Treatment and follow-up

Complete tumor staging was performed prior to NK-92 treatment. During cell infusion, patients were closely monitored, with vital signs recorded at 0, 15, 30, 60, 90, 120 and 240 min and every 24 h thereafter. Patients were examined daily for clinical toxicity from NK-92 infusion for the first 7 days and then weekly thereafter until 4 weeks after cell infusion. NCI-CTC version 3 criteria were used to document toxicities. CBC and chemistries were performed daily during the treatment course. CT scans were repeated at 2 and 4 weeks after the treatment course to assess disease response, and thereafter per routine by their local oncologist. Tumor response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) [12]. Additionally, a minor response was defined as regression of target tumor lesions by 10–30% with no new lesions and no non-target lesion progression. A mixed response was defined as the regression of some lesions but simultaneous progression of others.

Cytokine assays

Patient sera were collected pre-NK-92 cell infusion (time 0), at 4 h after each infusion on days 1, 3 and 5, and at 7 days post-infusion. The sera at each time point were tested by enzyme-linked immunosorbent assay (ELISA) with a standard multiplexed panel of cytokines (Linco Diagnostic Services Inc., St Charles, MI, USA). The cytokine panel consisted of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, interferon (IFN)-γ, granulocyte–macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF)-α. Four patients had cytokines measured at the higher NK-92 dose level with the hypothesis that the higher cell dose of NK-92 would tend to be more effective.

HLA antibody production

High-resolution DNA typing of the NK-92 cell line was used to establish its HLA type. High-resolution DNA typing for HLA was also performed on two patients for whom 1–2 year follow-up blood samples were available. The patient HLA class I and class II antibody (Ab) production against NK-92 was determined for these samples using standard cytotoxic cross-match and flow cytometric cross-match testing.

Statistical analysis

Analyzes were descriptive and graphical. Under the cytokine analysis, a one-sided sign-test was applied to the data from the four patients who had cytokines measured, to test the significance of the average of pre–post differences.

Results

Patient characteristics

The characteristics of the 12 patients enrolled in the study are summarized in Table 1. The median age was 50 years (range 31–74 years); eight patients were male and four were female. Eleven patients had refractory metastatic renal cell cancer, predominantly clear cell type. One patient had refractory metastatic melanoma, spindle cell type. Prior therapies included nephrectomy, high-dose IL-2, IFN, radiation, chemotherapy and SCT.

Table 1. Baseline characteristics of patients treated with NK-92 ($n = 12$)

Variable	Summary
Median age (years)	50 (range 31–74)
Gender	
Male	8
Female	4
Type of tumor	
Renal cell carcinoma	11
Melanoma	1
Metastatic sites	
Lung	10
Liver	4
Brain/central nervous system	1
Bone	3
Lymph nodes	6
Other	2
Prior therapies	
Surgery	11
IL-2, other immunotherapy (IFN, thalidomide)	10
Chemotherapy	3
Stem cell transplant	1
Radiation	4
Vaccine	1

Toxicity

All 12 patients received the three infusions of NK-92 per protocol and there were no delays in the infusion days. Table 2 summarizes the NK-92-related toxicities during the treatment course. Three patients (patients 8, 9 and 12) experienced grade 1 fevers (range 38.2–38.7°C) during the course of NK-92 infusion and all occurred with the higher dose level of $1 \times 10^9/m^2$. The fevers were self-limited and did not require treatment. The patient with metastatic melanoma developed a temperature of 41°C 4 h after the third infusion of NK-92, which responded to hydrocortisone 100 mg intravenously (i.v.). Blood and urine cultures, as well as culture of the NK-92 bag, were negative. This patient had new onset softening of his bulky pre-auricular and occipital tumor masses with frank drainage from the pre-auricular mass as it softened. There were no serious infections reported for patients at the 1-year follow-up post-NK-92 infusion.

Toxicities that were attributed to the underlying tumor and unrelated to NK-92 infusion included grade 2 neck and chest pains and grade 3 back pain in a patient with bulky retroperitoneal renal cell cancer. One grade 4 hypoglycemic episode (glucose $<$ 20 mg/dL) with symptoms of confusion and seizure-like activity occurred immediately after the first NK-92 infusion in a non-diabetic patient (11) who had extensive liver metastases. The patient's baseline glucose was normal at 162 mg/dL. The hypoglycemia responded to D50 bolus followed by continuous D5 i.v. infusion overnight. No further hypoglycemia episodes occurred with the subsequent two NK-92 infusions.

Clinical outcomes

The follow-up on this study is now 4 years, with all patients followed until death. Patients were allowed to seek other therapies after the 4-week toxicity monitoring period. As a phase I study, the study was not designed to evaluate formally the tumor response or duration of response. One patient (6) had a transient mixed response during the monitoring period. She had extensive metastases in the bilateral lungs, hila, mediastinum, abdominal and retroperitoneal nodes. The mixed response occurred as progression in the mediastinum but reduction in lung masses. She ultimately progressed and died at day 168 post-treatment. Patient 10, with melanoma, had a minor response in a target lesion at the left upper neck that was documented at 2 weeks post-infusion by physical examination and CT scan (Figure 1a,b). This patient, with very advanced disease, subsequently progressed and received alternative therapy, but did survive to 255 days post-NK therapy. Of the 12 patients who completed NK-92 treatment, 11 have subsequently died, 10 from progressive disease. Patient 3, who underwent reduced-intensity allogeneic sibling-matched transplant subsequent to NK-92 treatment, died 2.5 years later from consequences of the post-transplant immunosuppressed state, with bronchopneumonia and no active renal cell cancer. Patient 7 is the only surviving patient post-NK-92 infusion. He had progression at 4 weeks post-NK-92 infusion and went on to receive salvage therapies as allowed by the protocol. He was alive with disease and seeking further therapy for renal

Table 2. Adverse events in patients receiving NK-92 infusions. The severity of adverse events was graded according to NCI-CTC version 3

Subject	Diagnosis	Cell dose/m^2 × 3 doses	Adverse event w/grade (possibly related)
1	RCC	1×10^8	0
2	RCC	1×10^8	0
3	RCC	1×10^8	0
4	RCC	3×10^8	0
5	RCC	3×10^8	0
6	RCC	3×10^8	0
7	RCC	1×10^9	0
8	RCC	1×10^9	1, fever
9	RCC	1×10^9	1, fever
10	Melanoma	3×10^9	3, fever
11	RCC	3×10^9	4, hypoglycemia
12	RCC	3×10^9	1, fever

RCC, renal cell cancer.

Table 3. Clinical outcomes

Subject	Diagnosis	Cell dose/m^2 × 3 doses	Outcome at 4 weeks	Deaths (unrelated to NK-92)
1	RCC	1×10^8	PD*	D1006, PD
2	RCC	1×10^8	PD	D101, PD
3	RCC	1×10^8	PD†	D832, bronchopneumonia
4	RCC	3×10^8	PD	D666, PD
5	RCC	3×10^8	PD	D188, PD
6	RCC	3×10^8	Mixed	D168, PD
7	RCC	1×10^9	PD	Alive D1450
8	RCC	1×10^9	SD	D212, PD
9	RCC	1×10^9	SD†	D1059, PD
10	Melanoma	3×10^9	MR	D255, PD
11	RCC	3×10^9	SD	D695, PD
12	RCC	3×10^9	SD	D466, PD

*RCC, renal cell cancer; PD, progressive disease; SD, stable disease; MR, minor response; D, day. *prior alloSCT; †subsequent alloSCT.*

cell cancer at the latest follow-up, on day 1450 post-NK-92.

Laboratory findings

There was a trend of LDH elevations that occurred with NK-92 infusion at the higher cell dose level of $1 \times 10^9/m^2$ (Figure 2). Patient 8 went from a baseline LDH of 185 U/L to 1269 U/L (normal 200–650 U/L) after the first NK-92 infusion, peaked at 2157 U/L after the third infusion, and remained elevated through day 7 (1493 U/L). Patient 11, with the hypoglycemic episode, had a dramatic increase in her serum LDH to 1219 U/L at 4 h after the first NK-92 infusion. The LDH remained elevated through the subsequent two infusions, 1536 and 1254 U/L, respectively, but normalized at day 14 of the treatment course to 237 U/L. Patient 10, with metastatic melanoma, who developed high-grade fever and a clinical tumor response, similarly had elevation from a baseline normal LDH of 409 U/L to a peak of 791 U/L and 763 U/L on infusion days 3 and 5, respectively, with ultimate normalization to 327 U/L at day 14.

Other laboratory parameters examined did not show clinically significant changes in total WBC, platelets, neutrophil count, lymphocyte count or eosinophil count in patients over the three NK-92 infusions or in the 4 weeks of follow-up.

Cytokines were measured in four of the higher cell dose patients' sera pre-, at 4 h post- each of the three NK-92

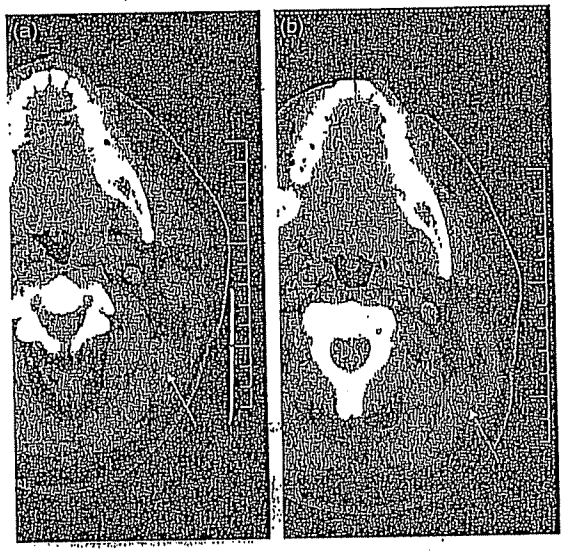

Figure 1. (a) Patient 10, pre-NK-92 infusion, left upper neck mass, 3.15 × 2.54 cm. (b) Two weeks post-NK-92 infusion, shrinkage of left upper neck mass, 2.46 × 1.76cm.

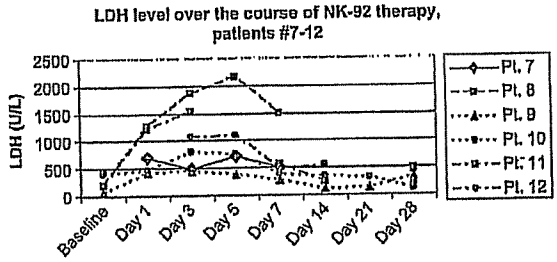

Figure 2. Trend of LDH elevation during NK-92 infusion starting at $1 \times 10^9/m^2$ cell dose. After an initial increase during treatment, the LDH values return to baseline by day 14.

infusions, and at 7 days post-infusion. Positive elevations in IL-6, IL-8 and IL-10 cytokines were seen with NK-92 infusion at the higher cell doses, perhaps suggesting tumor lysis. In patient 10, with metastatic melanoma, clinical tumor shrinkage correlated with a massive rise in IL-6, to 6819 pg/mL from a baseline of 17 pg/mL, along with grade 3 fever. IL-8 and IL-10 similarly rose (Table 4) and then normalized by day 7 post-infusion. Another observation was in patient 9, with metastatic renal cell cancer, who had baseline elevations of IL-4, IL-6 and IL-8, possibly reflecting constitutive cytokine secretion from the renal tumor.

As only four patients had cytokines measured, the sample size limited the degree of statistical reliability. However, if the IL-6, IL-8 and IL-10 pre–post differences (three per patient) are averaged within patients, in all four patients the average pre–post difference was always positive. This has a one-sided sign-test *P*-value of 0.0625, which is the smallest *P*-value obtainable in a non-parametric test with only four patients.

High-resolution HLA typing for NK-92 was confirmed as follows: A3, A11; B7, B44, Bw4$^+$, Bw6$^+$; Cw*07(3R), Cw*1601(3R); DR7, DR15; DQ2, DQ6; DR51$^+$, DR52$^-$, DR53$^+$. Samples from two patients (1 and 11) were tested for the development of anti-HLA Ab against NK-92. Patient 1 was found to have both HLA class I and class II Ab to the NK-92 cell line at 2 years post-exposure. Cytotoxicity and flow cytometric cross-match assays were also positive for this patient. For patient 11, panel reactive Ab and cross-match assays were negative at 1 year post-exposure.

Discussion

The development of the continuously growing NK-92 as a universal donor of highly cytotoxic tumoricidal cells is attractive for allogeneic cellular immunotherapy. Renal cell cancer and melanoma were chosen as the target diseases for this trial based on their previously reported immune responsiveness as tumors [2–4].

The main objective of the phase I trial was to determine the feasibility and safety of administration of NK-92 cell therapy with multiple infusions in these advanced cancer patients. NK-92 cells were successfully expanded under GTP conditions, on average 200–fold over 15–17 days with $\geq$80% viability. Infusional toxicities were generally minimal, limited to grade 1 fevers. No severe hemodynamic or hematologic toxicities were seen with the NK-92 infusion, and thus it compares favorably with other cellular immunotherapies that have used autologous NK or allogeneic haplo-identical NK cells [13–18].

The two major toxicities of grade 3 fever and grade 4 hypoglycemia seen in two patients, while temporally

Table 4. Serum cytokine measurements pre- and post-NK-92 doses. Cytokines were measured in the patients' sera before, 4 post- each of the three NK-92 infusions and at 7 days post-NK-92 infusion. Elevations in IL-6, IL-8 and IL-10 cytokines were seen with NK-92 infusion in the sample of four patients at the higher cell doses, with return to baseline by day 7

Patient	Diagnosis	Cell dose/ $m^2 \times 3$ doses	NK-92 infusion no.	IL-6* (pg/mL) Pre-	Post-	Day 7	IL-8* (pg/mL) Pre-	Post-	Day 7	IL-10* (pg/mL) Pre-	Post-	Day 7
8	RCC	1×10^9	1	34	71		5	15		<3	<3	
			2	215	94		11	6		<3	4	
			3	125	214	35	9	12	10	<3	<3	<3
9	RCC	1×10^9	1	282	307		339	298		41	22	
			2	291	276		257	327		7	74	
			3	284	286	282	299	309	305	7	24	9
10	Melanoma	3×10^9	1	17	18		20	24		<3	<3	
			2	46	29		27	19		66	44	
			3	17	6819	14	20	607	15	<3	159	<3
11	RCC	3×10^9	1	4	13		25	37		42	906	
			2	<3	<3		15	19		32	327	
			3	<3	<3	<3	16	21	31	19	190	96

**The one-sided sign test has a P-value of 0.0625 for the average of pre–post differences.*

related to the NK-92 infusions, could be reflective of tumor lysis responses in these large tumor burden patients versus a reaction to the infusion of cells. The hypoglycemic response in patient 11, who had extensive liver metastases, could be related to tumor-induced hypoglycemia, which has been described in patients with extensive liver metastases [19]. Such a response could be the result of the release of insulin or a humoral hypoglycemic factor, such as an insulin-like substance or diminished glycogen stores in the liver from extensive metastases [19], or ectopic hormone production by the primary renal tumor, such as IGF-2, that can cause hypoglycemia [20]. Hypoglycemia in this setting might also be interpreted as a surrogate for a tumor lysis reaction [21], as may the increase in LDH seen in several patients after infusion of NK-92 . LDH increase is rather non-specific, however, and one cannot rule out other possibilities for the rise in LDH, such as from dead or dying NK-92 cells that were irradiated prior to infusion.

Similarly, elevations in IL-6, IL-8 and IL-10 with NK-92 infusion at the higher cell doses might suggest tumor lysis reaction. However, the cancers themselves can express these cytokines, as can the NK-92 cell line or a toxic response to the infusion of the cells, making it difficult to interpret the cytokine responses in a small sample of patients.

One patient developed HLA Ab whereas another did not. This result may point to a variability in the immune response to NK-92, and this may in part be explained by the variable host immunocompromised status. Other factors to consider are that prior blood product transfusions in the patient could induce an alloimmune response that is cross-reactive with those Ag expressed by NK-92. A larger number of patients will need to be studied to answer this issue. Still, there would seem to be a logical approach in avoiding retreatment of patients having a positive cross-match beyond a 7-day window in order to prevent an anamnestic response.

The exact mechanism of NK-92 killing has not been established; however, it can be hypothesized that NK-92 essentially lacks KIR because of its immature status, and thus target killing is predominantly through its natural cytotoxicity receptors (NKp30 and NKp46) and activating receptor NKG2D [22], rather than a KIR-mediated NK alloreactivity mechanism. The clinical advantage may be that allogeneic NK cellular therapy with NK-92 has a broader spectrum of tumor killing because it overcomes the 'self' MHC molecule restriction, much as has been hypothesized for adoptive transfer of haplo-identical NK cells in patients with cancer [18,23].

Efficacy was not determined in this phase I trial; however, there were two patients with changes in tumor measurement that seemed to meet minor and mixed responses during the study period. These changes were, as expected, transient in this heavily pretreated population. Having determined the safety of infusion and feasibility of large-scale expansion in this initial study, the future plans with NK-92 include a phase II study to determine the biologic activity in other advanced cancers, and to draw on its unique advantage as a cell line to be a platform for genetic engineering to target tumor Ag, such as ErbB2 [24] and CD20 [25], to increase the potential for improved tumor localization and killing efficacy.

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Infusion of the allogeneic cell line NK-92 in patients with advanced renal cell cancer or melanoma: a phase I trial

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Background

Renal cell cancer and malignant melanoma are two types of cancer that are responsive to immunotherapy. In this phase I dose-escalation study, the feasibility of large-scale expansion and safety of administering ex vivo*-expanded NK-92 cells as allogeneic cellular immunotherapy in patients with refractory renal cell cancer and melanoma were determined.*

Methods

Twelve patients (aged 31–74 years) were enrolled, three per cohort at cell dose levels of $1 \times 10^9/m^2$, $3 \times 10^9/m^2$, $1 \times 10^9/m^2$ and $3 \times 10^9/m^2$. One treatment course consisted of three infusions. Eleven patients had refractory metastatic renal cell cancer; one patient had refractory metastatic melanoma.

Results

The NK-92 cells were expanded in X-Vivo 10 serum-free media supplemented with 500 U/mL Proleukin recombinant human interleukin-2 (rhIL-2), amino acids and 2.5% human AB plasma. Final yields of approximately 1×10^9 cells/culture bag (218–250 × expansion) over 15–17 days were achievable with ≥ 80% viability. Infusional toxicities of NK-92 were generally mild, with only one grade 3 fever and one grade 4 hypoglycemic episode. All toxicities were transient, resolved and did not require discontinuation of treatment. One patient was alive with disease at 4 years post-NK-92 infusion. The one metastatic melanoma patient had a minor response during the study period. One other patient exhibited a mixed response.

Discussion

This study establishes the feasibility of large-scale expansion and safety of administering NK-92 cells as allogeneic cellular immunotherapy in advanced cancer patients and serves as a platform for future study of this novel natural killer (NK)-cell based therapy.

Keywords

cancer, cell therapy, NK-92, phase I.

Introduction

Treatment options remain very limited for patients with metastatic renal cancer and metastatic melanoma. Median survival is 7–10 months for metastatic renal cancer and metastatic melanoma and both diseases are resistant to chemotherapy and/or radiotherapy [1]. Both cancers, however, seem to be responsive to immunotherapy [2–4] and cellular immunotherapy is increasingly being considered as a form of treatment that is non-cross-reactive with prior chemotherapy and radiation [5,6].

Natural killer (NK) cells are particularly attractive for adoptive cellular immunotherapy because of their unique ability to lyse target cells without priming [7]. Autologous NK cells from cancer patients, however, may be dysfunctional and may not recognize the malignant target. Autologous NK cells may also be inhibited by 'self' HLA expression and some tumors may in fact express functional HLA antigens (Ag) capable of inhibiting NK cell function. Allogeneic NK cells, therefore, potentially represent a better NK cell product for immunotherapy. NK-92 is a human NK-cytotoxic cell line that represents a pure allogeneic activated NK cell source. NK-92 is interleukin-2 (IL-2) dependent, lacks killer cell inhibitory receptors (KIR) and is broadly cytotoxic against a variety of hematologic and solid tumor cell lines, including leukemia, lymphoma, malignant melanoma, prostate cancer and

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breast cancer [8]. *Ex vivo* expansion of NK-92 under good tissue practice (GTP) conditions for clinical use has allowed its entry into phase I study as a novel immunotherapy in advanced cancers [9]. The NK-92 cell line is originally derived from a non-Hodgkin's lymphoma with large granular lymphocyte morphology and a $CD56^+CD3^-CD16^-$ immunophenotype. Studies in SCID mice have confirmed that NK-92 inoculation itself is not leukemogenic. The tumoricidal activity of NK-92 against human leukemias has been tested *in vitro* against leukemic cell lines and primary leukemia cells, as well as *in vivo* by adoptive transfer of NK-92 cells into xenografted SCID mice, with the result of prolonged survival and no signs of leukemia development [10]. NK-92 infusion has further been found to prolong survival in SCID mice inoculated with human malignant melanoma cells, an observation that served as the basis for this clinical trial [11].

The objective of this study was to determine the safety of infusing NK-92 cells in patients with advanced renal cell cancer and melanoma. The three infusions, each given 48 h apart, had no severe side-effects and several patients showed objective anti-tumor responses, suggesting further exploration of this cellular treatment modality in selected cancer indications is warranted.

Methods

Patient eligibility

The study was open from April 2002 to June 2004 at Rush University Medical Center (Chicago, IL, USA). The protocol was approved by the Institutional Review Board and had obtained FDA investigational new drug application status for the *ex vivo* expansion of NK-92 cells. All patients signed informed consent before any study-related procedures. Patients with histologically confirmed metastatic renal cell cancer or malignant melanoma refractory to, or having failed, standard therapy, including surgery, radiation and chemotherapy, were eligible for treatment on this protocol. All patients had measurable disease [by computed tomography (CT) scan or physical examination] and had undergone several prior treatments, including high-dose IL-2 therapy and allogeneic stem cell transplant (SCT). Other eligibility criteria included ECOG 0 or 1, white blood cells (WBC) $> 2.0 \times 10^9$/L, Hb > 8 g/dl, platelets $\geq 75 \times 10^9$/L, creatinine < 2.0 mg/dL and total bilirubin < 2.0 mg/dL. Exclusion criteria included ECOG ≥ 2 and concurrent treatment with corticosteroids and/or other immunosuppressive drugs.

Trial design

The trial was a single-center, open-label, dose-escalation study. Three patients were treated at each dose level: 1×10^8 cells/m^2, 3×10^8cells/m^2, 1×10^9 cells/m^2 and 3×10^9cells/m^2. One treatment course consisted of three infusions of the cell dose over 48 h. Infusion days were designated as days 1, 3 and 5. The rationale for the schedule was to infuse as many NK-92 cells before a T-cell directed immune response would theoretically occur.

Manufacturing of the NK-92 cell product

Manufacturing of clinical-grade NK-92 cells was performed under GTP conditions at the Sramek Center for Cell Engineering at Rush University Medical Center [9]. At 3 weeks before the targeted date of infusion, NK-92 cell cultures were initiated from the NK-92 Working Cell Bank. NK-92 cells were expanded in X-Vivo 10 serum-free medium supplemented with 500 U/mL Proleukin recombinant human (rh)IL-2, 0.6 mm l-asparagine, 3 mm l-glutamine, 1.8 mm l-serine and 2.5% human AB plasma. The cultures were initiated at 2.5×10^5 cells/mL in 25 mL (6.25×10^6 cells) in 1-L Vuelife culture bags (American Fluoroseal Corp., Gaithersburg, MD, USA), with the addition of media every 3 days, maintaining a density of 2.5×10^5 cells/mL, and with daily mild disruption of cell aggregates. Final yields of approximately 1×10^9 cells/culture bag (218–250-fold expansion) over 15–17 days was achievable, with $\geq 80\%$ viability. After quality control verification and quality assurance release that included Gram stain, culture and mycoplasma testing, the final NK-92 cell product was resuspended in GM-2 medium (Plasma-Lyte-A medium supplemented with 2.5% human AB plasma) and infused fresh. The last feeding with rhIL-2 and fresh medium was 48 h before the first day of infusion of the expanded NK-92 product. In addition, after completion of the cell culture period, a standard cytotoxicity assay was performed to assess the functional capacity of the *ex-vivo*-expanded NK-92 cells. Calcein AM-labeled K562 and Raji cells were used as targets to determine NK-92 cell cytotoxicity of the *ex vivo*-expanded cells. The NK-92 cells were irradiated with 1000 cGy prior to infusion into the patient (Cesium Source-Blood Bank, Rush University Medical Center).

On the day of infusion, hydration (200 mL NS/h) was given to the patient 2 h prior to the NK-92 cell infusion and continued for 2 h after NK-92 infusion. The total volume of the NK-92 cell product infusate was

100–200 mL, depending on the body weight of the individual patient. The cells were infused at a rate of 5 mL/min, with a total infusion time of approximately 20–30 min. All patients received premedication with diphenhydramine before the start of each cell infusion.

Of note, the NK-92 cell line was being commercialized during the course of the clinical trial.

Treatment and follow-up

Complete tumor staging was performed prior to NK-92 treatment. During cell infusion, patients were closely monitored, with vital signs recorded at 0, 15, 30, 60, 90, 120 and 240 min and every 24 h thereafter. Patients were examined daily for clinical toxicity from NK-92 infusion for the first 7 days and then weekly thereafter until 4 weeks after cell infusion. NCI-CTC version 3 criteria were used to document toxicities. CBC and chemistries were performed daily during the treatment course. CT scans were repeated at 2 and 4 weeks after the treatment course to assess disease response, and thereafter per routine by their local oncologist. Tumor response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) [12]. Additionally, a minor response was defined as regression of target tumor lesions by 10–30% with no new lesions and no non-target lesion progression. A mixed response was defined as the regression of some lesions but simultaneous progression of others.

Cytokine assays

Patient sera were collected pre-NK-92 cell infusion (time 0), at 4 h after each infusion on days 1, 3 and 5, and at 7 days post-infusion. The sera at each time point were tested by enzyme-linked immunosorbent assay (ELISA) with a standard multiplexed panel of cytokines (Linco Diagnostic Services Inc., St Charles, MI, USA). The cytokine panel consisted of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, interferon (IFN)-γ, granulocyte–macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor (TNF)-α. Four patients had cytokines measured at the higher NK-92 dose level with the hypothesis that the higher cell dose of NK-92 would tend to be more effective.

HLA antibody production

High-resolution DNA typing of the NK-92 cell line was used to establish its HLA type. High-resolution DNA typing for HLA was also performed on two patients for whom 1–2 year follow-up blood samples were available. The patient HLA class I and class II antibody (Ab) production against NK-92 was determined for these samples using standard cytotoxic cross-match and flow cytometric cross-match testing.

Statistical analysis

Analyzes were descriptive and graphical. Under the cytokine analysis, a one-sided sign-test was applied to the data from the four patients who had cytokines measured, to test the significance of the average of pre–post differences.

Results

Patient characteristics

The characteristics of the 12 patients enrolled in the study are summarized in Table 1. The median age was 50 years (range 31–74 years); eight patients were male and four were female. Eleven patients had refractory metastatic renal cell cancer, predominantly clear cell type. One patient had refractory metastatic melanoma, spindle cell type. Prior therapies included nephrectomy, high-dose IL-2, IFN, radiation, chemotherapy and SCT.

Table 1. Baseline characteristics of patients treated with NK-92 ($n = 12$)

Variable	Summary
Median age (years)	50 (range 31–74)
Gender	
Male	8
Female	4
Type of tumor	
Renal cell carcinoma	11
Melanoma	1
Metastatic sites	
Lung	10
Liver	4
Brain/central nervous system	1
Bone	3
Lymph nodes	6
Other	2
Prior therapies	
Surgery	11
IL-2, other immunotherapy (IFN, thalidomide)	10
Chemotherapy	3
Stem cell transplant	1
Radiation	4
Vaccine	1

Toxicity

All 12 patients received the three infusions of NK-92 per protocol and there were no delays in the infusion days. Table 2 summarizes the NK-92-related toxicities during the treatment course. Three patients (patients 8, 9 and 12) experienced grade 1 fevers (range 38.2–38.7°C) during the course of NK-92 infusion and all occurred with the higher dose level of $1 \times 10^9/m^2$. The fevers were self-limited and did not require treatment. The patient with metastatic melanoma developed a temperature of 41°C 4 h after the third infusion of NK-92, which responded to hydrocortisone 100 mg intravenously (i.v.). Blood and urine cultures, as well as culture of the NK-92 bag, were negative. This patient had new onset softening of his bulky pre-auricular and occipital tumor masses with frank drainage from the pre-auricular mass as it softened. There were no serious infections reported for patients at the 1-year follow-up post-NK-92 infusion.

Toxicities that were attributed to the underlying tumor and unrelated to NK-92 infusion included grade 2 neck and chest pains and grade 3 back pain in a patient with bulky retroperitoneal renal cell cancer. One grade 4 hypoglycemic episode (glucose $<$ 20 mg/dL) with symptoms of confusion and seizure-like activity occurred immediately after the first NK-92 infusion in a non-diabetic patient (11) who had extensive liver metastases. The patient's baseline glucose was normal at 162 mg/dL. The hypoglycemia responded to D50 bolus followed by continuous D5 i.v. infusion overnight. No further hypoglycemia episodes occurred with the subsequent two NK-92 infusions.

Clinical outcomes

The follow-up on this study is now 4 years, with all patients followed until death. Patients were allowed to seek other therapies after the 4-week toxicity monitoring period. As a phase I study, the study was not designed to evaluate formally the tumor response or duration of response. One patient (6) had a transient mixed response during the monitoring period. She had extensive metastases in the bilateral lungs, hila, mediastinum, abdominal and retroperitoneal nodes. The mixed response occurred as progression in the mediastinum but reduction in lung masses. She ultimately progressed and died at day 168 post-treatment. Patient 10, with melanoma, had a minor response in a target lesion at the left upper neck that was documented at 2 weeks post-infusion by physical examination and CT scan (Figure 1a,b). This patient, with very advanced disease, subsequently progressed and received alternative therapy, but did survive to 255 days post-NK therapy. Of the 12 patients who completed NK-92 treatment, 11 have subsequently died, 10 from progressive disease. Patient 3, who underwent reduced-intensity allogeneic sibling-matched transplant subsequent to NK-92 treatment, died 2.5 years later from consequences of the post-transplant immunosuppressed state, with bronchopneumonia and no active renal cell cancer. Patient 7 is the only surviving patient post-NK-92 infusion. He had progression at 4 weeks post-NK-92 infusion and went on to receive salvage therapies as allowed by the protocol. He was alive with disease and seeking further therapy for renal

Table 2. Adverse events in patients receiving NK-92 infusions. The severity of adverse events was graded according to NCI-CTC version 3

Subject	Diagnosis	Cell dose/m^2 × 3 doses	Adverse event w/grade (possibly related)
1	RCC	1×10^8	0
2	RCC	1×10^8	0
3	RCC	1×10^8	0
4	RCC	3×10^8	0
5	RCC	3×10^8	0
6	RCC	3×10^8	0
7	RCC	1×10^9	0
8	RCC	1×10^9	1, fever
9	RCC	1×10^9	1, fever
10	Melanoma	3×10^9	3, fever
11	RCC	3×10^9	4, hypoglycemia
12	RCC	3×10^9	1, fever

RCC, renal cell cancer.

Table 3. Clinical outcomes

Subject	Diagnosis	Cell dose/m^2 ×3 doses	Outcome at 4 weeks	Deaths (unrelated to NK-92)
1	RCC	1×10^8	PD*	D1006, PD
2	RCC	1×10^8	PD	D101, PD
3	RCC	1×10^8	PD†	D832, bronchopneumonia
4	RCC	3×10^8	PD	D666, PD
5	RCC	3×10^8	PD	D188, PD
6	RCC	3×10^8	Mixed	D168, PD
7	RCC	1×10^9	PD	Alive D1450
8	RCC	1×10^9	SD	D212, PD
9	RCC	1×10^9	SD†	D1059, PD
10	Melanoma	3×10^9	MR	D255, PD
11	RCC	3×10^9	SD	D695, PD
12	RCC	3×10^9	SD	D466, PD

*RCC, renal cell cancer; PD, progressive disease; SD, stable disease; MR, minor response; D, day. *prior alloSCT; †subsequent alloSCT.*

cell cancer at the latest follow-up, on day 1450 post-NK-92.

Laboratory findings

There was a trend of LDH elevations that occurred with NK-92 infusion at the higher cell dose level of $1 \times 10^9/m^2$ (Figure 2). Patient 8 went from a baseline LDH of 185 U/L to 1269 U/L (normal 200–650 U/L) after the first NK-92 infusion, peaked at 2157 U/L after the third infusion, and remained elevated through day 7 (1493 U/L). Patient 11, with the hypoglycemic episode, had a dramatic increase in her serum LDH to 1219 U/L at 4 h after the first NK-92 infusion. The LDH remained elevated through the subsequent two infusions, 1536 and 1254 U/L, respectively, but normalized at day 14 of the treatment course to 237 U/L. Patient 10, with metastatic melanoma, who developed high-grade fever and a clinical tumor response, similarly had elevation from a baseline normal LDH of 409 U/L to a peak of 791 U/L and 763 U/L on infusion days 3 and 5, respectively, with ultimate normalization to 327 U/L at day 14.

Other laboratory parameters examined did not show clinically significant changes in total WBC, platelets, neutrophil count, lymphocyte count or eosinophil count in patients over the three NK-92 infusions or in the 4 weeks of follow-up.

Cytokines were measured in four of the higher cell dose patients' sera pre-, at 4 h post- each of the three NK-92

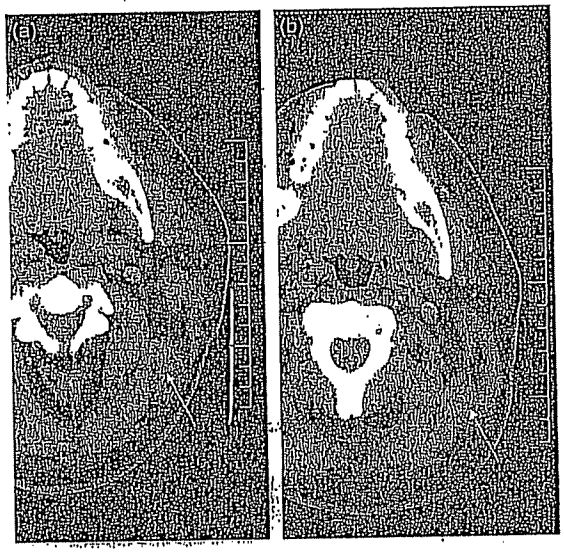

Figure 1. (a) Patient 10, pre-NK-92 infusion, left upper neck mass, 3.15 × 2.54 cm. (b) Two weeks post-NK-92 infusion, shrinkage of left upper neck mass, 2.46 × 1.76cm.

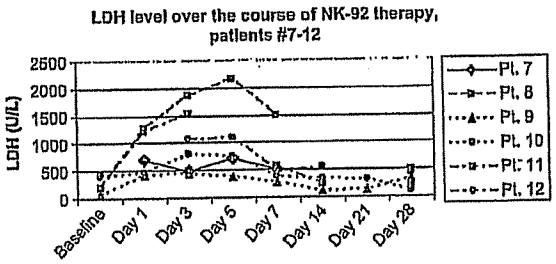

Figure 2. Trend of LDH elevation during NK-92 infusion starting at $1 \times 10^9/m^2$ cell dose. After an initial increase during treatment, the LDH values return to baseline by day 14.

infusions, and at 7 days post-infusion. Positive elevations in IL-6, IL-8 and IL-10 cytokines were seen with NK-92 infusion at the higher cell doses, perhaps suggesting tumor lysis. In patient 10, with metastatic melanoma, clinical tumor shrinkage correlated with a massive rise in IL-6, to 6819 pg/mL from a baseline of 17 pg/mL, along with grade 3 fever. IL-8 and IL-10 similarly rose (Table 4) and then normalized by day 7 post-infusion. Another observation was in patient 9, with metastatic renal cell cancer, who had baseline elevations of IL-4, IL-6 and IL-8, possibly reflecting constitutive cytokine secretion from the renal tumor.

As only four patients had cytokines measured, the sample size limited the degree of statistical reliability. However, if the IL-6, IL-8 and IL-10 pre–post differences (three per patient) are averaged within patients, in all four patients the average pre–post difference was always positive. This has a one-sided sign-test *P*-value of 0.0625, which is the smallest *P*-value obtainable in a non-parametric test with only four patients.

High-resolution HLA typing for NK-92 was confirmed as follows: A3, A11; B7, B44, Bw4$^+$, Bw6$^+$; Cw*07(3R), Cw*1601(3R); DR7, DR15; DQ2, DQ6; DR51$^+$, DR52$^-$, DR53$^+$. Samples from two patients (1 and 11) were tested for the development of anti-HLA Ab against NK-92. Patient 1 was found to have both HLA class I and class II Ab to the NK-92 cell line at 2 years post-exposure. Cytotoxicity and flow cytometric cross-match assays were also positive for this patient. For patient 11, panel reactive Ab and cross-match assays were negative at 1 year post-exposure.

Discussion

The development of the continuously growing NK-92 as a universal donor of highly cytotoxic tumoricidal cells is attractive for allogeneic cellular immunotherapy. Renal cell cancer and melanoma were chosen as the target diseases for this trial based on their previously reported immune responsiveness as tumors [2–4].

The main objective of the phase I trial was to determine the feasibility and safety of administration of NK-92 cell therapy with multiple infusions in these advanced cancer patients. NK-92 cells were successfully expanded under GTP conditions, on average 200–fold over 15–17 days with $\geq$80% viability. Infusional toxicities were generally minimal, limited to grade 1 fevers. No severe hemodynamic or hematologic toxicities were seen with the NK-92 infusion, and thus it compares favorably with other cellular immunotherapies that have used autologous NK or allogeneic haplo-identical NK cells [13–18].

The two major toxicities of grade 3 fever and grade 4 hypoglycemia seen in two patients, while temporally

Table 4. Serum cytokine measurements pre- and post-NK-92 doses. Cytokines were measured in the patients' sera before, 4 post- each of the three NK-92 infusions and at 7 days post-NK-92 infusion. Elevations in IL-6, IL-8 and IL-10 cytokines were seen with NK-92 infusion in the sample of four patients at the higher cell doses, with return to baseline by day 7

				IL-6* (pg/mL)			IL-8* (pg/mL)			IL-10* (pg/mL)		
Patient	Diagnosis	Cell dose/ $m^2 \times 3$ doses	NK-92 infusion no.	Pre-	Post-	Day 7	Pre-	Post-	Day 7	Pre-	Post-	Day 7
8	RCC	1×10^9	1	34	71		5	15		<3	<3	
			2	215	94		11	6		<3	4	
			3	125	214	35	9	12	10	<3	<3	<3
9	RCC	1×10^9	1	282	307		339	298		41	22	
			2	291	276		257	327		7	74	
			3	284	286	282	299	309	305	7	24	9
10	Melanoma	3×10^9	1	17	18		20	24		<3	<3	
			2	46	29		27	19		66	44	
			3	17	6819	14	20	607	15	<3	159	<3
11	RCC	3×10^9	1	4	13		25	37		42	906	
			2	<3	<3		15	19		32	327	
			3	<3	<3	<3	16	21	31	19	190	96

**The one-sided sign test has a P-value of 0.0625 for the average of pre–post differences.*

related to the NK-92 infusions, could be reflective of tumor lysis responses in these large tumor burden patients versus a reaction to the infusion of cells. The hypoglycemic response in patient 11, who had extensive liver metastases, could be related to tumor-induced hypoglycemia, which has been described in patients with extensive liver metastases [19]. Such a response could be the result of the release of insulin or a humoral hypoglycemic factor, such as an insulin-like substance or diminished glycogen stores in the liver from extensive metastases [19], or ectopic hormone production by the primary renal tumor, such as IGF-2, that can cause hypoglycemia [20]. Hypoglycemia in this setting might also be interpreted as a surrogate for a tumor lysis reaction [21], as may the increase in LDH seen in several patients after infusion of NK-92 . LDH increase is rather non-specific, however, and one cannot rule out other possibilities for the rise in LDH, such as from dead or dying NK-92 cells that were irradiated prior to infusion.

Similarly, elevations in IL-6, IL-8 and IL-10 with NK-92 infusion at the higher cell doses might suggest tumor lysis reaction. However, the cancers themselves can express these cytokines, as can the NK-92 cell line or a toxic response to the infusion of the cells, making it difficult to interpret the cytokine responses in a small sample of patients.

One patient developed HLA Ab whereas another did not. This result may point to a variability in the immune response to NK-92, and this may in part be explained by the variable host immunocompromised status. Other factors to consider are that prior blood product transfusions in the patient could induce an alloimmune response that is cross-reactive with those Ag expressed by NK-92. A larger number of patients will need to be studied to answer this issue. Still, there would seem to be a logical approach in avoiding retreatment of patients having a positive cross-match beyond a 7-day window in order to prevent an anamnestic response.

The exact mechanism of NK-92 killing has not been established; however, it can be hypothesized that NK-92 essentially lacks KIR because of its immature status, and thus target killing is predominantly through its natural cytotoxicity receptors (NKp30 and NKp46) and activating receptor NKG2D [22], rather than a KIR-mediated NK alloreactivity mechanism. The clinical advantage may be that allogeneic NK cellular therapy with NK-92 has a broader spectrum of tumor killing because it overcomes the 'self' MHC molecule restriction, much as has been hypothesized for adoptive transfer of haplo-identical NK cells in patients with cancer [18,23].

Efficacy was not determined in this phase I trial; however, there were two patients with changes in tumor measurement that seemed to meet minor and mixed responses during the study period. These changes were, as expected, transient in this heavily pretreated population. Having determined the safety of infusion and feasibility of large-scale expansion in this initial study, the future plans with NK-92 include a phase II study to determine the biologic activity in other advanced cancers, and to draw on its unique advantage as a cell line to be a platform for genetic engineering to target tumor Ag, such as ErbB2 [24] and CD20 [25], to increase the potential for improved tumor localization and killing efficacy.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Cell Biology

ATCC® Number:	**CRL-2407™**	Order this Item	Price: **$281.00**

Designations:	NK-92
Depositors:	ZelleRx Corporation
Biosafety Level:	1 [Human]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension, multicell aggregates
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast
Source:	**Disease:** malignant non-Hodgkin's lymphoma **Cell Type:** natural killer cell; NK cell;
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (technology from amaxa)
Antigen Expression:	CD2 +, CD7 +, CD11a +, CD28 + , CD45 +, CD54 +, CD56 +, CD1 -, CD3 -, CD4 -, CD5 -, CD8 -, CD10 -, CD14 -, CD16 -, CD19 -, CD20 -, CD23 -, CD34 -, HLA-DR -
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9,12 D16S539: 11,12 D5S818: 12,13 D7S820: 10,11 THO1: 6,9.3 TPOX: 8 vWA: 18
Age:	50 years
Gender:	male
Ethnicity:	Caucasian, White

NK-92 is an interleukin-2 (IL-2) dependent Natural Killer Cell line derived from peripheral blood mononuclear cells from a 50 year old Caucasian male with rapidly progressive non-Hodgkin's lymphoma. [38894]
The cell line is dependent on the presence of recombinant Il-2 and a dose as low as 10 U/ml is sufficient to maintain proliferation; cells will die within 72 hours in the absence of IL-2. [38894]
The cell line is cytotoxic to a wide range of malignant cells; it

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Comments:	kills both K562 cells and Daudi cells in chromium release assays. [38894] NK-92 cells (after irradiation to prevent proliferation) can be used effectively for immunological ex vivo purging of leukemia from blood without compromising hematopoietic cell function. [38896] NK-92 cells have the following characteristics: surfacemarker positive for CD2, CD7, CD11a, CD28, CD45, CD54 and CD56 bright; surface marker negative for CD1, CD3, CD4,CD5, CD8, CD10, CD14, CD16, CD19, CD20, CD23, CD34 and HLA-DR. [38894]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is Alpha Minimum Essential medium without ribonucleosides and deoxyribonucleosides but with 2 mM L-glutamine and 1.5 g/L sodium bicarbonate . To make the complete growth medium, add the following components to the base medium: 0.2 mM inositol; 0.1 mM 2-mercaptoethanol; 0.02 mM folic acid; 100-200 U/ml recombinant IL-2; adjust to a final concentration of 12.5% horse serum and 12.5% fetal bovine serum. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** Successful growth of this cell line is very dependent upon the quality of IL-2 used in the growth medium. ATCC recommends using the highest quality IL-2 available.
Subculturing:	**Protocol:** Cultures can be maintained by addition or replacement of medium. When replacing media, centrifuge cells and resuspend cell pellet in fresh medium at 2 to 3 X 10 (5) viable cells/ml. Pipet the cells up and down on the back of the flask every 2-3 days to produce a single cell suspension. NK-92 cells are extremely sensitive to overgrowth and media exhaustion. **Medium Renewal:** Replace with fresh medium every 2 to 3 days (depending on cell density)
Preservation:	**Freeze medium:** 50% FBS; 40% complete growth medium ; 10% DMSO. **Storage temperature:** liquid nitrogen vapor phase
Related Products:	recommended serum:ATCC 30-2020 recommended serum:ATCC 30-2040 derivative:ATCC CRL-2408 derivative:ATCC CRL-2409

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X. RELATED PROCEEDINGS APPENDIX

(1) U.S. Patent Application No. 10/701,359, filed on November 4 2003, entitled "Methods of Treating Tumors Using Natural Killer Cell Lines," which is a divisional of the '955 Application, which is a continuation-in-part of U.S. Patent Application No. 09/403,910, filed on October 27, 1999, now abandoned, which is a national phase entry of PCT/US98/08672, filed on April 30, 1998 and which claims priority to U.S. Provisional Application No. 60/045,885, now expired, filed on April 30, 1997.

(2) U.S. Patent Application No. 10/456,237, filed on June 6, 2003, entitled "Interleukin-Secreting Natural Killer Cell Lines and Methods of Use," which is a divisional of the '955 Application, which is a continuation-in-part of U.S. Patent Application No. 09/403,910, filed on October 27, 1999, now abandoned, which is a national phase entry of PCT/US98/08672, filed on April 30, 1998 and which claims priority to U.S. Provisional Application No. 60/045,885, now expired, filed on April 30, 1997.